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BUTTER-FAT (GHEE)

Its Composition, Nutritive Value, Digestibility,
Rancidity, Adulteration,
Its Detection and Estimation.

BY

N. N. GODBOLE

AND

SADGOPAL



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Preface to the Second Edition.

The first edition of this treatise was sold out within a few years of its publication. Obviously, it seems to have served a longfelt want. The second edition has long been over-due. The number of enquiries for the new edition, received both in India and from out-side, has been very large. One of the main causes of this delay in publication has been the absence from India of Dr. Godbole who went over to Japan in the latter half of the year 1937.

The size of the second edition has, the authors are glad, increased substantially, almost to five times the size of the first edition. The causes for this increase have been many. A few years ago, Mahatma Gandhi sent round an enquiry all over India, inviting the opinion of all interested in the subject of "the comparative utility of the milk and butter-fat of cows and buffaloes" as a part of his rural re-construction programme. Our detailed note sent to Mahatmaji on this subject received his special commendation and a special mention has been made of this fact in one of the issues of "Harijan". As a result of this, a number of inquiries were received by us from all parts of India and therefore, we made up our mind to include this information also in our new edition. Along with the food value of Ghee, the desire for the information of other food-stuffs, both vegetarian and non-vegetarian, has increased in India of late. The calorific value of the daily need of the average Indian was also one of the subjects of inquiry and the information collected both in India and also in Japan (in 1937) has been included in this edition.

The old and new standards of the purity of Ghee fixed by the Indian Government have been reproduced together with our criticisms of the same. The old standards would be of great value for later references as also for showing the evolution of a subject of all-India importance. The evolution of the different methods adopted in Ghee-analysis have been specially discussed in one separate chapter because some of our critics have suggested certain old values as being equivalent to or as being even better than the latest values suggested and worked upon by us. A special exhaustive chapter has been added on "the rancidity of Butter-fat"—a work which has been recently completed by Mr. Sadgopal—because, in some cases, in India certain rancid samples have been wrongly pronounced as adulterated by experts and also because under Indian conditions, rancidity sets in comparatively easily. It is hoped, this information will be welcome to many.

Very valuable experimental data and results included in this edition have been lent by Sadgopal from his thesis submitted for the Doctorate degree and this is very gratefully to be acknowledged here.

The importance attached to the subject of Ghee and the references made to it and to its uses in the Vedas, the oldest scriptures of India are naturally read with interest and admiration by many. We are thankful to Pt. S. D. Satawalekar for his having supplied to us the following valuable extracts.

- १ घृतमन्नम् । ऋ० १० । ९९ । २; २ । ३५ । ११; २ । ३५ । १४
- २ शूष्यं घृतम् । ऋ० ५ । ८६ । ६
- ३ शूषं घृतम् । ऋ० ६ । १० । २
- ४ अन्नस्य घृत मेव रसस्तेजः । मंत्र ब्रा० २ । ६ । १५

५ तेजोवा एतत्पशूनां यद् घृतम् ॥ ऐ० ब्रा० ८ । २०

६ रेतो वै घृतम् । श० ब्रा० ९ । २ । ३ । ४४ वा यजु० १७ । ७९

In राजनिघंटु (वर्ग १५), the properties of fresh Ghee and good Ghee as a tonic and as a nourishing food have been described. The use of very old (rancid) Ghee is recommended for certain medicinal purposes. Further, in अत्रि संहिता (९), special mention is made of how in certain cases of fever, dyspepsia, indigestion, etc, the use of Ghee is to be avoided. This knowledge is of great academic interest and shows the advance made by ancient India.

August, 1939.

Benares Hindu University.

N. N. GODBOLE,

SADGOPAL.

Preface to the first edition.

Of all the articles of food, Butterfat or Ghee occupies, to the vegetarian in India, a very important position in the daily diet. Its importance is fully emphasised in the ancient Vedic Text

“ आयुर्वै घृतम् ”

which puts Ghee as being the equivalent of “Life” itself. Carbohydrates, albuminoids and fats are the three types of food, next to salts and vitamins, absolutely essential for the uniform and proportionate development of the human body. For the *vegetarian* in India, any variety of material is available to supply the necessary quantity of carbohydrates and albuminoids : but so far as the supply of fats is concerned, there is no other source of material except pure Ghee (of animal origin !) and vegetable oils. This explains the importance of the Vedic text given above. The adulteration of Ghee is only of recent origin ; adulterants ¹ such as cottonseed oil, coconut oil and groundnut oil etc., are being freely used for some time. Latterly, however, in the imported vegetable Ghee, the sophisticator has had a handy substance which has got all the external properties of Butter Ghee. This adulteration is freely practised because of the complexity of the chemical composition of Ghee and the difficulties it presents in its analysis and detection. On enquiring from the market, we learn that the degree of adulteration varies, according to the market conditions, from about five per cent to almost cent per cent. With the growth of scientific knowledge, the adulteration is being carried

(1) Bolton E. R. and Revis C.-Some analysis of Ghee, *Analyst*, 1910, 35, 343-346.

on so systematically and intelligently that its detection also is now developing into a special branch of analytical science. It is, therefore, very difficult to devise a simple and, if possible, a domestic test to detect the adulteration as many a layman desires to do. In every civilised country (except India!), the manufacture of Butter is a question of public hygiene and as such is subject to Government and Municipal control which makes the crime of adulteration punishable by law. In Berlin, for example, there is a regular State Laboratory in which a specially qualified staff is systematically working to detect all crimes of this nature. Owing to the very close relations existing between the Universities and the state laboratories, a considerable portion of research work is being carried on by the University students, in this subject, in the "Material-Prüfungs-Amt."

The continued application of the Food Laws in England¹ has had such a beneficial effect that in one case of an annual examination of as many as 15,124 samples of butter, only 867 were found to be adulterated *i.e.*, to say the adulteration amounted to only five per cent. It is remarkable to note that even in this five per cent, the actual percentage of adulteration did not exceed a maximum of only 15 per cent. We have learnt from wholesale merchants in the seaport towns of India—as a contrast with the above statement—that the adulteration has gone on to such a limit that pure vegetable ghee is being sold under the name of butter-ghee.

Apart from the question of food value (to which we shall refer later), ordinary courtesy demands that the purchaser should be fully acquainted with the nature of the material he is buying and that he should get his money's worth. The purchaser has a right to get

¹) Encyclopaedia Britannica, 11th edition, article on Adulteration.

the genuine stuff and the State is morally bound to help him. The demand for the adoption of measures by the Government to prevent the adulteration of Ghee with the imported vegetable ghee (as also of other adulterants) has been very insistent and wide-spread. The need for legislation in this direction is, therefore, reasonable, urgent and overdue. A clear exposition of the nature and composition of these articles is a primary necessity. It then becomes a responsible part of the duties of the Minister in charge of Public Hygiene and Sanitation to issue regulations from time to time on the subject and make the crime of adulteration punishable by Law.

The adulteration of Butterfat has now become a serious menace to public health. Not only are the natural oils and fats being used with impunity but even fats obtained from snakes and other diseased animals¹ are being fraudulently mixed.

The task of the analyst has been rendered very difficult by the incompleteness of the methods in vogue today. The methods known so far are applicable to and are drawn from European and American conditions. Butter, as it is, keeps long enough in all cold countries and, therefore, its conversion into the dehydrated form *i.e.*, Ghee is not known except in certain warm zones of Europe. The question of the correct understanding of Ghee, therefore, becomes typically a question for all tropical countries, a parallel question in Europe being that of Margarine *vs.* Butter. The need, therefore, of devising a rapid and reliable method becomes obvious.

As we were receiving a number of enquiries on this subject and as we found out that the existing information in India was both meagre and insufficient, we made up our mind to take up the work as a piece of research on Oils

(1) Watt, G. : Commercial Products of India, p. 479.

and Fats (in which subject this Department is specially interested); more so because we felt that being a typically Indian question it should interest Indians primarily. Our work extending over a period of nearly one year is being embodied in the following pages, in which we have tried to make a critical survey of the literature available on the subject both in India and outside and have suggested our own methods for the solution of the problem. We have fully censored our methods and are satisfied with the accuracy of our observations which we have embodied in the form of tables and graphs. We have no doubt, they can be repeated elsewhere if they are carried out under proper supervision. The work of investigation is still being carried on and it is expected that more interesting results will follow.

The present work has been divided into three parts as follows. The first part concerns itself with the discussion of the composition, the nutritive value and the digestibility of the butterfat and also deals with the discussion of the limitations involved in the known methods of oil and fat analysis. The second part deals with the comparative study of the methods known and employed hitherto for the above purpose. In part three, we have proposed and discussed the new methods supported by our experimental data and have shown their practical utility both for qualitative and quantitative examinations. We have fully drawn upon all the sources of information available on the subject and have duly acknowledged our gratefulness to them. We have recorded our criticism in a scientific spirit and we shall be thankful to those interested in the subject for a similar criticism. The problem is one of all-India importance and fully deserves the attention of many more willing workers.

Vijayadashami, (October, 1930)

Benares.

N. N. GODBOLE,
SADGOPAL.

**Some press opinions and private appreciations,
of the first edition.**

1. **American Perfumer and Essential Oil Review,**
New York, April, 1931, Page 99.

“Any one interested in the chemistry of glyceride oils and fats should find this pamphlet of interest. The work covers not only Butterfat but also fats and oils used as substitutes in adulterating it. In this respect, the information will be of great interest.....*The work is brief, clearly written and the data is well-assembled.* The results are charted on a graph which is included in the appendix. *It is a constructive contribution to the literature of oils and fats.*”

2. **Hindu, Madras, 4th January, 1931.**

“The authors have summarised the results arrived at by modern investigators both in Europe and in America and have, in the course of their summary, pointed out the unsatisfactory nature of the results, so far as the problem in India is concerned..... The method suggested applies both for qualitative and quantitative analysis; by its adoption, one could not only state roughly the percentage of adulteration but also the nature of the adulterant. We welcome the publication of this new method and hope that it will be tried in the Indian municipalities with a view to take action and to prevent the sale of adulterated ghee in India.”

3. **Allgemeine Oel und Fett-Zeitung, Berlin, Special
Margarine Industry Number, 25th April, 1931,
Page 151.**

“N. N. Godbole and Sadgopal have carried out a *thorough investigation* regarding the detection of adultera-

tion in Butterfat. The most important result arrived at by them is based on the observation that adulteration of foreign fats in butterfat can be detected by the colour-fringes which are observed in the Wollny Butyro-refractometer. In the case of pure ghee, the fringe is light violet, in the case of coconut oil, it is orange-red and in the case of vegetable ghee and other fats, it is bluish green. If, instead of diffused sunlight, direct sunlight is used in the refractometer, the colour-fringe, the authors find, becomes very sharp. For the quantitative estimation, the authors have used the exact determination of the A and B values which have been *suitably modified by them for their special work.*"

4. Statesman, Calcutta, 27th December, 1930.

"A reminder of this practice of adulteration (of Ghee) comes today in the form of a booklet issued by Prof. Dr. N. N. Godbole and Mr. Sadgopal of the Department of Industrial Chemistry, Benares Hindu University, who suggest new methods by which adulteration may be detected. While of special interest to the municipalities, the scientist, the doctor and the public health worker, their thesis may profitably be read also by the layman if only because it will lead the latter to greater wariness."

5. Oil and Fat Industry, New York, October, 1931.

"The authors have undertaken a discussion of clarified butterfat (Ghee), from the standpoint of protection of the purity of this product which is so popular with the native Indian population The booklet contains a number of interesting tables of values determined by the authors."

6. Tribune, Lahore, 31st December, 1930.

"The pamphlet describes a new and a very accurate method for the qualitative and quantitative detection of

adulterants in Ghee. The pamphlet is well-planned and divided into three parts. The authors have demonstrated the unreliability of the existing methods for the detection of small quantities of adulterants in Ghee."

7. **Chemische Umschau, Stuttgart, 29th July, 1931,**
Page 228.

"In the first part, the authors have discussed the composition, the nutritive value and other properties of butterfat (Ghee); then in the second part, the methods known hitherto regarding the investigation of butterfat have been discussed. In the third part, the authors recommend the use of the Butyro-refractometer and the determination of the refractive-index and the estimation of A and B values for detecting adulteration. *The work is very comprehensive and is provided with many instructive tables.*"

8. **Leader, Allahabad, 16th February, 1931.**

"This pamphlet should prove useful not only to chemists, analysts, doctors, municipal laboratories and Government analytical laboratories but also to ghee merchants and the general public who are interested in the general problem of ghee and its adulteration. The printing and get-up are all that could be desired."

9. **British Soap Manufacturer, London,**
September, 1931, Page 203.

"Graphs and tables of refractive indices are included and the references to related scientific work are numerous and very complete. *The work is recorded in a scientific spirit and criticism is welcomed as the problem is one of all-India importance. It is a very creditable contribution, well-arranged and neatly presented.*"

10. United India and Indian States, Delhi,
31st January, 1931.

“It is a *valuable* handbook describing the *original research* in a subject that is of primary importance to Indian life. We congratulate the authors in tackling such a highly significant matter in its purely scientific aspect and releasing for publication their considered views after close study and research. We recommend this book to all chemists, physiologists and hygienists interested in human welfare. The experimental records and bibliography show the amount of work done and its practical importance.”

11. Seifensieder-Zeitung, Augsburg, 26th February, 1931,
Page 133.

“In the closing chapter, the Indian researchers have given a complete list of the literature which they have consulted and *it is surprising with what thoroughness they have utilised these references for their purpose*. No less than thirty-seven independent authors have been consulted—amongst whom, we find the following Germans, Lunge, Holde, Gruen, Ullmann, Witzoeff and a long list of English and German technical journals.”

12. Scientific Indian, Calcutta, December, 1930,
Page 377.

“We are convinced that the collaborators have *closely considered all the known methods* of physical and chemical analysis of butterfat. They have applied and tested the accuracy of their proposed methods critically. In order to appreciate the *thoroughness and patience* with which the experiments have been carried out, suffice it to mention that as many as forty-five different samples of ghee from different parts of India have been tested. *The authors deserve the encomium of all ghec-lovers* for having

contributed to the technical literature of this country a *treatise which is unique in many respects.*"

13. **British Medical Journal, London, January, 1932,**
Page 66.

"The authors review the current chemical and physical methods for estimating the constituents of mixtures of fats, find them all unsatisfactory and propose a method of their own, depending on the use of a refractometer".

14. **Industry, Calcutta, January, 1931.**

"The chief merit of the book consists in the fact that these tests which usually require some technical knowledge have been described in such a *novel and simple* manner that the adulteration may be detected easily. . . . We hope that the little book will no doubt remove the difficulties usually felt by analytical chemists in determining the amount of foreign substances incorporated with ghee".

15. **Searchlight, Patna, 28th December, 1930.**

"The authors have done a great service to the country and to science by publishing this pamphlet in which has been given a new and a very accurate method for the qualitative and quantitative detection of adulterants in ghee. The authors are to be congratulated on the production of such a *valuable and suggestive* pamphlet. There is, so far as is known, *no other pamphlet of its type in existence.*"

16. **Late Geheimrat Prof. Dr. D. Holde, Technische Hochschule, Berlin, 6th June, 1931.**

"My best thanks for your monograph about "The nourishing value, adulteration, detection and estimation of butterfat". I am very happy that you have dealt with this problem of the valuation and estimation of the "Equivalent of life" in such a *thorough manner and that*

you have collected your material from the most varied sources available. I am happy that in doing so *you have awakened in India the consciousness of the knowledge* of the reliable and correct composition of this most important food material”.

17. Hindu Herald, Lahore, 12th January, 1931.

“Dr. N. N. Godbole and Mr. Sadgopal of the Benares Hindu University have done a service to the country by doing a piece of research work on oils and fats. . . . This treatise on its nutritive value, adulteration, detection and estimation will enable the public to understand a subject of which they ought to know more. The book is *exhaustive and contains every information* available on the subject. The authors have taken elaborate pains to put down methods. . . . The book will be useful to all who are interested in the national welfare of India”.

18. Prof. Dr. H. P. Kaufmann, Muenster.

I. W. University, 6th July, 1931.

“I have read your book about butterfat with great interest. The pamphlet which is *very carefully written* and which *comprehensively covers an amount of authentic literature receives my full approval*”.

19. Kesari, Poona, 18th April, 1931.

“In writing the pamphlet, the authors have tapped a large number of English, French and German authors. We believe, *this is the only and the first book of its kind* which has incorporated so much information about ghee. We suggest it to the authors that *the pamphlet should be translated in all the Indian provincial languages.*”

20. Professor Dr. Ubbelohde, Karlsruhe (Germany),

2nd February, 1931.

“I thank you very heartily for your sending me a copy of your pamphlet about butterfat. I have gone

through it and I am glad to state that a *very critical investigation of the subject* has been made. I shall embody the researches in the next edition of my "Handbuch" (of Oil and Fat technology). It will be a great pleasure to me to receive from you further communications on the subject; for I am particular that in my (Five volumes of) "Handbuch", not merely European but all really good researches from the whole world should be incorporated."

21. The Aj, Benares, 8th January, 1931.

"This pamphlet is *highly useful to all* those who are engaged in the investigation of the analysis and the adulteration of butterfat. We recommend this to every one who is interested in the knowledge of butterfat. The value of this work would be enhanced if it were written in Hindi instead of in English for which (latter) the number of readers would naturally be limited (in India)."

22. Dr. E. Lewkowitsch, London, 1st July, 1932.

"I was interested to see that you have attained *such satisfactory results* by the use of the Bertram A and B method which I think has not attracted yet the interest it deserved; at least in this country but little notice has been taken of it....I shall be very interested to hear the results of the further researches foreshadowed in your thesis."

23. Prof. Dr. W. Halden, University of Graz (Germany),
11th September, 1934.

"As the quantitative estimation of the adulteration of butterfat is by no means an easy or simple problem, the authors have done a great service to science by developing *a reliable method* for testing Indian ghee, a method that also might prove useful for examining butterfats of other countries. Therefore it is to be expected that the second

enlarged edition of the *valuable booklet will also find the full approval of the international chemists working on food-stuff-analysis.*"

24. The Director of Dairy Research Institute (N. Z.),
Palmerston North, 16th August, 1935.

"I am very much obliged to you for sending us this booklet which is *most interesting and valuable.*"

25. Late Prof. Dr. E. Gildemeister, Goslar (Germany),
6th July, 1934.

"I thank you very much for your sending me a copy of your very interesting treatise on "Butterfat".

26. Prof. Dr. Ing. J. Tausz, Karlsruhe (Germany),
13th February, 1931.

"I thank you very much for a copy of the very interesting book on Butterfat."

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CHAPTER I.

Composition of Milk, Comparative values and Utility of Milk of different animals including human Milk.

Elsewhere we have discussed the comparative merits and utility of the two butter-fats, obtained from the cow and the buffalo, as human food materials. A similar study of the milk of these two animals along with that of some others is now proposed to be made in the following paragraphs.

In every civilized country of the world, an adequate national food policy presupposes an abundant supply of milk to foster the healthy growth of every individual in the nation. In India particularly, milk has been an indispensable article of human diet. It is necessary, therefore, to study scientifically the comparative food value of the milk as obtained from the cow and the buffalo. We have also included in this note, the study of the milks of certain other animals as also mother's milk in order to throw proper light on the subject, and to study their comparative importance.

According to Ullmann, "Milk is the product obtained from the milk glands of female animals from the time of the birth of the young ones as required for *their* nourishment. It contains to a sufficient degree all the materials necessary for the building up and the growth of the various animals." Ost, another standard author gives another exhaustive definition: "Milk is an emulsion of fats in which milk sugar and albumin and certain inorganic salts are dissolved along with casein in the form of calcium

caseinate in a colloidal form. The emulsification of the milk fat resembles the emulsion obtained from a mixture of solution of soap with animal oil or fat. It is this state of fine emulsion which renders it so easily digestible.”

Milk is primarily made up of the following constituents : (a) water, (b) fat, (c) albuminoids consisting of the difficultly digestible casein and easily digestible lactalbumin, (d) milk sugar and (e) salts consisting of phosphates, citrates and chlorides of metals like calcium, potassium and sodium besides (f) enzymes like Oxidase and Catalase. Vitamins also form a very important part of the whole composition.

From the above, it is naturally clear that the milk of different animals contains all those constituents which are necessary for a systematic growth of the particular young ones, in desirable proportions. These young ones get *doubled* in their weights by living simply on the mother’s milk, in the following periods.

Table No. 1.

No.	Animal.	Number of days required to double the weights.
1	Hare	6 Days.
2	Sheep	15 ..
3	Cow	50 ..
4	Horses	60 ..
5	Children	180 ..

For a proportionate and healthy growth of the human body, normal food should consist of (a) carbohydrates, (b) fats and (c) albuminoids in certain definite proportions. Salts, vitamins and spices are also indispensable to activate the whole growth. Spices help digestion besides increasing the appetite. One very rich source of salts is

vegetables which also act as "Excitants". The total heat required by the human body for being converted into work varies, not only according to the average work done by an individual, but also depends upon the difference between the temperature of the surrounding atmosphere and the normal temperature of the human body. In India, often we take for comparison the figures in terms of heat calories applicable to an average adult in Europe which is inaccurate.

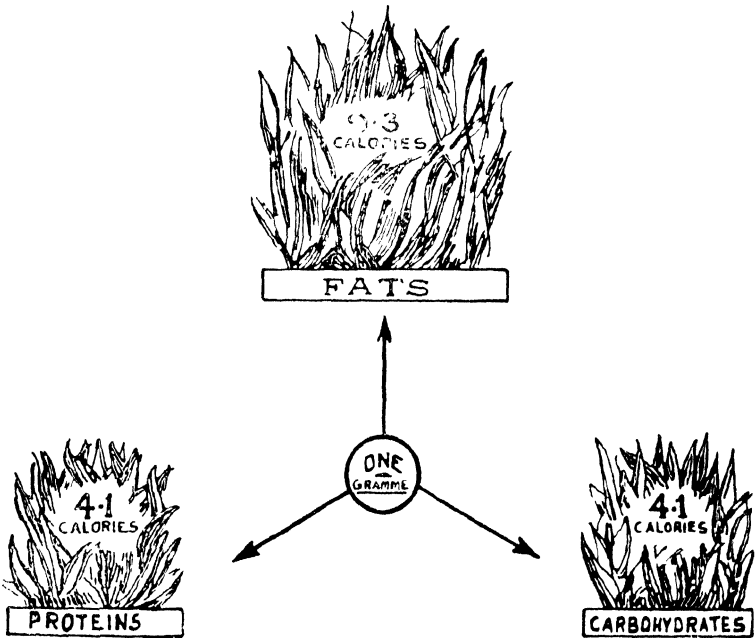
In a recent article published in the "Leader" of Allahabad, in April 1929, Prof. Dr. N. R. Dhar of the Allahabad University has quoted a recommendation made by the British Medical Association of England from which he estimates that we in India need about 3000 calories per day. For this, he recommends, "we need (a) one seer of carbohydrates per day in the form of rice, potatoes, and sugar etc. (2000 calories) (b) half a *chhatak* of fats in the form of butter, ghee and oil etc., (500 calories), (c) 2 *chhataks* of protein-matter in the form of "Dal", milk (about 3/4 of a seer per day) and, if possible (for non-vegetarians) two eggs (500 calories,) (d) fresh fruits for vitamin C and (e) leafy green vegetables and tomatoes etc., for vitamins A, B and C." All this sounds very reasonable from an academic point of view. From a practical point of view, however, the question is, can we digest or assimilate all this quantity of food in those parts of India where the external atmospheric temperature is very much above the normal temperature of the body for several months in the year as in Rajputana, Sind, Punjab, U. P. and C. P. ? We are afraid, we cannot digest even half of this food, and if loaded, the human body will be more injured than benefitted. It is possible that in winter months, in certain parts of India such as U. P., the Punjab, Kashmir etc., where the winter is severe, provided our adults

do as much work as our colleagues in Europe, we may need the above quantity of food. In our opinion, it is necessary to carry out experiments on basal metabolism under Indian climatic conditions, before any general conclusions can be drawn.

We are afraid, the information borrowed from European books has got to be used in India with very great caution. Compare and contrast, for example, the conditions in the European winter and an Indian summer. In the winter season, in Central Europe for instance, the temperature outside ranges between -10° to -20°C . (body temperature being $+37^{\circ}\text{C}$. or $+98.5^{\circ}\text{F}$.) In India, on the contrary, in summer, in parts like the U. P., Punjab and Sindh etc, a temperature of $+45^{\circ}\text{C}$. or 113°F is not uncommon. This means (taking two extremes into consideration) that in Europe, in winter, the external temperature is lower than the body temperature by about 50°C ., whereas, in India, in summer, the external temperature is higher than the body temperature by about 8°C . In Europe, therefore, one must feed himself in winter *even if he is doing no work* to make up for the loss due to radiation of heat, in order to maintain the body temperature at 37°C ., whereas in India in summer, the outer temperature being higher than the body temperature, one has to exert to keep the body temperature lower than the surrounding temperature. This is enough to prove that one has to be extremely cautious in applying the European data concerning food, work and calories of heat etc., to Indian conditions.

According to accepted physiological laws, the amount of food taken is meant to serve two purposes; firstly to maintain the normal temperature of the body and secondly to replace the constituents of the body used up in doing actual work. So far as Indian conditions are concerned,

HEAT ENERGIES
OF
FATS, PROTEINS - AND CARBOHYDRATES.



one gram of fats yields 9.3 calories.

one gram of Proteins yields 4.1 Calories.

one gram of Carbohydrates yields 4.1 Calories.

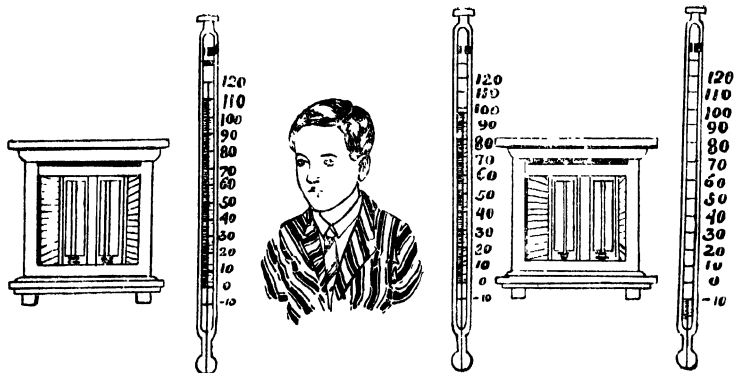
Body Temperature and Surrounding Temperature

IX

India and Central Europe.

Body Temperature, in India, is *lower* than the surrounding temperature in summer and is *higher* than the surrounding temperature in winter.

In Central Europe, the body temperature is *higher* than the surrounding temperatures all the year round.



Body Temp.

98.4° F.

INDIA

Max. +120° F.

Min. + 35° F.

CENTRAL EUROPE

Max. + 90° F.

Min. - 20° F.

Plate No. 8.

pit is erfectly reasonable to suppose that during the summer months as well as in the rainy season, when the surrounding temperature is high, not much heat energy is required for radiation to keep the body temperature normal. In fact, in certain provinces where it is very hot, it is necessary to take cold drinks of very little food value to lower the body temperature externally and internally by allowing water to evaporate in the form of perspiration escaping through the large evaporating surface of the body like an earthenware jug of water.

To be brief, the calories of heat required for the daily work have got to be distributed between the carbohydrates, fat and albuminoids in the ratio of about roughly 5 : 1 : 1, all of which together supply the necessary amount of energy required by an adult. According to European authors, depending upon the weight of and work done by an adult, he needs 2500 to 3000 calories of heat energy per day. It is a pity there is so little data *available for Indian conditions*. It is worthwhile finding out, how much and what quality of food should be useful to a worker who taxes his brain more than his body. As is well-known, lecithin, a derivative of glycerides containing nitrogen and phosphorus is especially found in the brain and in the nerves. Food containing lecithin, for example eggs (for the non-vegetarians), soya-bean and some other leguminous beans (for the vegetarians), should be valuable for the brain-worker. From this point of view, milk is a very valuable food because it contains all the necessary ingredients both for the invalids as well as for the adults, particularly so for the former. An examination of the various types of milks from different animals will, therefore, be of very great interest.

(*Note.*—During my second visit to Japan in September 1937, I visited the Food Research Institute at Tokyo.

From the data available there, I have gathered that for Indian conditions a sum of 2000—2500 calories should suffice. Very valuable work has been done in Tokyo on the basal metabolism and during the months of July, August and September, conditions in Japan are almost similar to conditions in India. The Japanese average of these months can be taken up as an average for India, as given above.—N. N. GODBOLE).

Table No. 2.

Average composition of different milks from foreign samples.

...	Sp. gravity.	Water %	Protein %	Fat %	Sugar %	Ash. %	Total Solids %
Woman ..	1.0298	87.58	2.01	3.71	6.37	0.30	12.42
Cow ..	1.0313	87.27	3.39	3.68	4.94	0.72	12.73
Ass ..	1.0320	90.12	1.85	1.37	6.19	0.47	9.88
Sheep ..	1.0355	83.57	5.15	6.18	4.17	0.93	16.43
Goat ..	1.0305	86.88	3.76	4.27	4.64	0.85	13.12
Mare ..	1.0347	90.58	2.05	1.14	5.87	0.36	9.42
Sow ..	1.0380	83.49	7.23	4.55	3.23	1.05	16.06
Bitch ..	1.0350	75.44	11.17	9.57	3.69	0.73	24.56
Elephant ..	1.0313	79.30	2.51	9.10	8.59	0.50	20.70
Camel ..	1.0420	86.57	4.00	3.07	5.59	0.77	13.43
Buffalo (Egyptian)	1.0350	82.25	5.05	7.51	4.44	0.75	17.75
Buffalo (Indian)	1.0298	81.92	4.25	7.55	4.75	0.89	18.08

Table No. 3.

Composition of various milks (according to Godbole-Sadgopal) from different animals.

No.	Origin.	Total Solids.	Albumi- no'ids.	Fat.	Milk Sugar.	Ash.	Density.
1	Cow (average of about 50 samples) ..	12.9-14.5	3.4-4.0	3.0-3.85	4.5-5.2	0.45-0.65	1.026-1.035
2	Goat (average of 23 samples) ...	12.6-13.2	3.6-6.2	3.2-3.95	4.0-5.3	0.6-0.82	1.03-1.036
3	Sheep (average of 13 samples) ..	15.5-19.5	5.8-7.5	5.8-7.5	4.5-5.0	0.6-1.3	1.035-1.042
4	Buffalo (average of 63 samples) ..	18.0-22.5	5.3-6	6.5-3.75	5.0-5.4	0.7-0.95	1.038-1.042
5	Mare (average of 6 samples) ...	9.5-11.2	2.1-2.55	0.6-1.8	6.8-5	0.3-0.4	1.03-1.038
6	Ass (average of 7 samples) ...	9.16-9.53	1.6-2.0	1.3-1.5	6.28-6.8	0.4-0.48	1.023-1.035
7	Mother (average of 11 samples) ..	11.5-13.5	1.0-1.65	2.0-3.65	5.8-6.5	0.15-0.25	1.03-1.034
8	Elephant (average of 2 samples) ..	20.0-28.9	10.3-13.4	12.5-15.6	7.2-10.3	1.2-2.7	1.2-1.75
9	Pig (one sample) ..	21.7	8.6	9.78	6.5	1.9	1.403
10	Bitch (one sample) ..	14.6	4.7	4.2	3.8	0.52	1.038

From the above tables, it is clear that the constituents vary from one animal to another, depending upon the nature of the animal and its requirements for the growth of its young ones. Again even in the case of the same animal, it has been found that the constituents vary in their percentage contents, according to the requirements of its young ones, at different periods of its growth. It will be also seen from the above table, that in the case of hardy animals which have to do a lot of rough work, as is the case with the mare and the ass, the percentage of fat is always low.

The selection of a particular milk is to be made depending upon whether the adult in question requires more percentage of fat or of other constituents. From this point of view, it is desirable to study the problem with special reference to the individual constituents.

Specific Gravity :—This property is the net result of the presence of the various constituents in their percentage proportions. But this is never a very reliable and constant property. The various milks can be generally classified from this point of view as follows :

Table No. 4.

A comparison of the Specific Gravities of various milks according to Godbole-Sadgopal.

Elephant.	Pig.	Buffalo.	Sheep.	Bitch.	Mare.	Goat.	Cow.	Mother.	Ass.
1·2-1·75	1·403	1·030- 1·042	1·035- 1·042	1·038	1·03- 1·042	1·03- 1·036	1·026- 1·035	1·03- 1·034	1·023- 1·035

It will be interesting to note that the specific gravity of the milk increases on skimming off the lighter fat portion and therefore an extra addition of water is possible as

adulteration, the resulting mixture giving the correct specific gravity figures within the range of those of pure milk.

Colour of Milk :—It is commonly believed that the colour of cow's milk is yellow and that of the buffalo is what is known as "Milky-white". We very much doubt and question the accuracy of this statement. As is wellknown, milk is an emulsion of very finely divided particles whose size is also an important factor in determining the particular colour of the milk as studied from the point of view of colloidal chemistry. Again some of the colouring matter is also derived from green grasses etc., on which the animals are fed. It is this colouring matter from the grass etc., which gives a tinge to the milk and through the milk to the butter also. But this is only accidental. It has nothing to do with any physiological reactions involved in the building of milk in the system of the animal. If instead of straw or grass, the cow is fed on oilseed cakes, as is not very uncommon, the emulsion is white and not coloured. The yellow colour associated with cow's milk and cow's butter or ghee has almost become a superstition and as a consequence even the dairies use a yellow colouring matter in the manufacture of butter. The mere presence of a yellowish colour should not, therefore, mislead anybody in drawing an inference that necessarily cow's milk or butter is involved.

According to Neumann, winter butter is generally very nearly white because straw is added to the cake food given to the animal. If the animal is fed on carrots and other grasses, a yellowish colour is imparted to the butter (which is then known as "May-butter" "Summer-butter" or "Grass-butter") and a peculiar aroma also.

Fat content:—The nature of the different fats obtainable from various milks differs in every case according to the nature of the animal, climatic conditions, feeding and idiosyncrasy of the animal etc. A detailed study of the two fats as obtained from the milks of the cow and the buffalo has been made in a separate chapter. The classification of the fat-content by percentage is given in the following table.

Table No. 5.

A comparison of the fat-contents of various milks according to Godbole-Sadgopal,

Elephant	Pig	Buffalo.	Sheep.	Bitch.	Goat.	Cow.	Mother.	Ass.	Mare.
12.5-15.6	9.78	6.5-8.75	5.2-8.6	4.2	3.2-3.95	3.0-3.85	2-3.65	1.3-1.5	0.6-1.8

The milks of the American and European cows contain a lower percentage of fat than that of the Indian cows and that is why it is said that, in the military and Government dairies in India, a portion of the fat from the milk is skimmed off and the cream removed with a view to bring down the percentage content to between 3 to 4 and then the milk is sold as “Full Natural Milk”. This also suggests a method by which the milk of the cow and the buffalo can be converted into a milk akin to mother’s milk by simply treating the milk in an Alpha Lavel Separator and skimming off a portion of the cream so as to bring the fat content of the milk under consideration to that of mother’s milk. The removal of the extra fat will correspondingly raise the content of the proteins and salts in the milk, both of which are very desirable for the growth of the young ones. A subsequent addition of milk sugar is then made to make it equal to mother’s milk.

Milk Sugar :—This sugar is easily digestible and on fermentation gives acids like lactic and butyric etc. A small quantity of ethyl alcohol is also produced during the fermentation of this sugar as in the formation of Indian Curd or Dahi from milk. When milk gets sour, acids like lactic and butyric are produced. The digestive properties of the butter-milk are mostly dependent upon the lactic acid formed during the fermentative process and hence its importance in the Indian diet can be understood. The following table will show the order in which the various milks can be placed from the point of view of their percentage content of milk-sugar :

Table No. 6.

A comparison of the milk-sugar-content of various milks according to Godbole-Sadgopal.

Elephant.	Mare.	Ass.	Pig.	Mother.	Buffalo.	Sheep.	Cow.	Goat.	Bitch.
7·2-10·3	6·8·5	6·3-6·8	6·5	5·8-6·5	5·5·4	4·5·5	4·5·5·2	4·5·3	3·8

Albuminoids :—In general, it is known that the albuminoids of animal origin are more easily digestible than those from vegetable sources. For the same reason, the albuminoids present in Indian pulses are not so easily digestible as the nitrogenous matter from the milk-casein or the yolk of eggs. Again, the shorter the time taken by young ones to double their weights, the more the albuminous matter required for their growth. It is known that the greatest percentage of nitrogenous matter is to be found in the case of hare's milk, about 15·4 %. In the case of milk, we have got three types of proteins, (a) Casein, (b) Lacto-albumin and (c) Lacto-globulin. The latter two are more easily digestible by the human system

than the casein. Casein again differs in its nature according to the sources from which it is obtained. For example, it is known that the casein in the case of mother's milk is less easily precipitated than the other caseins, as in the case of elephant and buffalo's milks. Again the casein precipitated from mother's milk is much lighter than that of buffalo's or elephant's milk showing thereby that mother's milk contains casein which is more finely divided and hence, probably, more easily digestible. In the following table, the various milks are arranged according to their casein-content :

Table No. 7.

A comparison of the casein-content of various milks according to Godbole-Sadgopal.

Origin.	Elephant	Pig.	Buffalo.	Sheep.	Goat.	Bitch.	Cow.	Mare.	Ass.	Mother
% Casein	7.2-8.9	6.1	4.5-5.2	4.5-2	3.2-5.5	4.2	3-3.4	1.5-1.7	1.2-1.3	0.4-0.9
%Albumin + Globulin	3.1-4.5	2.5	0.8-0.95	1.8-2.3	0.4-0.7	0.5	0.4-0.6	0.6-0.85	0.4-0.7	0.6-0.75

These albuminoids play a very important role in the building up of the tissues of the human body for which very reason, some of the popular medicines for the purpose (as for example, Sanatogen) are rich in casein-content.

It is very interesting to note that the percentage of milk-sugar is very large in the case of mother's milk, although there is a deficiency in the percentage of albuminoids. It is, therefore, that children always like to take mother's milk as it tastes sweeter than the other milks which they are unwilling to take without some extra sugar.

Ash :—A study of the ash obtained by incinerating the solids and thus removing the organic matter is very interest-

ing. Salts of metals like calcium, magnesium, potassium, sodium, iron, containing chlorine, phosphorus and sulphur in a combined form are found to occur in various milks according to the nature of the animal and the food on which it is fed. Sulphur has been found only in the case of milks of the goat and the cow. Again, iron has been found only in the case of cow-milk while traces of iodine have been observed in both the milks of the cow and the goat. Physiologically speaking, these salts, though present in very minute quantities, play a very important part in resisting a number of diseases like T. B. and development of tonsillitis. In this connection, it should be remembered that due to the presence of these various salts of organic nature, the normal milk is amphoteric in its reaction *i.e.*, it behaves both as an acid and also as an alkali towards an indicator.

Vitamins :—Presence of vitamins in the milk is responsible to a very great extent for making the milk an excellent health tonic. According to the best authorities on the subject of vitamins, practically all the vitamins and especially vitamins A, D & E are abundantly present in the milks of various animals, the latter being prominent in the case of milks of the cow and the buffalo. In the case of the mother's milk, the vitamin potency varies according to the nature of the feeding. It is, therefore, that wise mothers always take special care of their food during the period of their pregnancy. Experimenters, in Europe, have found out that the vitamin-potency of mother's milk is practically nil in cases where the diet of the mothers consists of only *non-vegetarian menu*. Even in the case of other animals, it is found that vitamin-content is high in case of those animals which take the largest amount of greens, grasses and vegetables. A very strong argument in favour of milk is to be found in the

fact observed by American physicians that sterility in both sexes is generally found in those people who seldom take milk. Again, the vitamin potency of fresh milk of the cow and the buffalo (commonly known as घारोण्ण दुग्ध) and also of the fresh milk of the mother is the highest in comparison with the heated milks as the heat destroys many a vitamin. In India, children are given fresh milk (घारोण्ण दुग्ध) of goat by intelligent mothers and such children have been generally found to add to their growth very rapidly.

Taking all these points into consideration, there is no doubt that milk is an excellent food containing all the ingredients in *the proper proportions* required for healthy and proper growth of man. The ratio between the carbohydrates, fat and nitrogenous matter in the case of the following milks is very instructive :

Table No. 8.

Animal.	Goat.	Buffalo.	Cow.	Mother.	Ass.
Carbohydrates : Fat : Albuminoids.	1·5 : 1 : 1	1 : 1·5 : 1	1·5 : 1 : 1	5 : 2 : 1	5 : 1 : 1

The above calculation shows the superiority of ass's milk over mother's and that of the mother's over the cow's which in turn is better than all other milks as a human food material. It has been also quoted above that the chief constituents of food required in the case of a healthy human being should be in the ratio 5 : 1 : 1 approximately. It is extraordinary that nature should have been so very partial to an animal like the ass—that is so little trusted with common sense. Ass's milk is used in India as a special milk in the case of sickly children who cannot digest mother's milk.

The common usage of the expression "Bread and Butter" in Europe is most suited to denote an expressly good human diet. In India, we require a countrywide campaign of "Drink more Milk" in order to build up bonny babies and healthy adults who form the real backbone of a nation. During the war days in Germany, the supply of milk had to be thoroughly regulated due to the shortage of the rations. Preference used to be given to children and invalids over adults. Still, according to the then-Chief of German Board of Public Health, Prof. Drigalski, children in Germany were not healthy. "When sufficient quantities were once more available, the German children gained new vitality like withered flowers placed in water." That is enough to justify the importance of milk as a health-beverage.

From the medical point of view also, milk is very important besides possessing a high food-value. According to Hindu Medical Science, there is a regular system of treatment known as **पर्पटी** in which the patient is made to live for a period varying from twenty days to about a month and a half *only on milk*, for all his requirements. A very famous Vaidya, who is an authority on this subject, actually administers this system on patients with certain chronic diseases, otherwise given up as hopeless, and in most cases, rapid cures have been the result. In this treatment, in which an adult becomes once more a baby, milk is known to thoroughly cleanse up a diseased system and then it commences to rebuild the new tissues which give a new life to the patient. Physiologically, milk is known not to excite the nervous system or bodily organs and it avoids inflammation of kidneys.

Our conclusions are as follows :

(I) From the point of view of the contents of carbohydrates, fat, albuminoids, salts and vitamins, milk

especially of the mother, ass and cow form an ideal food both for adults and children.

(II) Of the two milks, of the cow and buffalo, the cow's is more easily digestible by the human system and hence is more nourishing.

(III) From the medicinal point of view, also, the milk of the cow and the goat are excellent.

The various scientific and physiological factors regarding the digestibility or the assimilability of cow's and buffalo's milks have been discussed above from an academic point of view. But in spite of all this, it should be remembered that there is such a thing as a temperament factor. It has come to our notice, that in many cases, as for example, where only buffalo's milk is available, those who are used to the taste of this milk have such a prejudice against the use or even the taste of cow's milk that they find cow's milk indigestible (?) in spite of the scientific fact that cow's milk ought to be very easily digestible when compared with buffalo's milk. Those, however, that are used to taking a large quantity of cow's milk are easily upset by even a small quantity of buffalo's milk, thanks to their preconceived prejudices and dislike for buffalo's milk. This factor being a question of individual likes and dislikes (स्वाद as it is called), as we have said elsewhere in connection with the use of edible oils, cannot possibly be discussed on scientific grounds. Like certain religious beliefs, such tastes do not stand the test of reason.

CHAPTER II

Physico-chemical constants and composition of the Butterfat of (a) cows and (b) buffaloes.

*Preparation of the samples of butterfat previous to their
being used for investigation.*

The butter used was made out of fresh natural milk. Wherever possible, the milk as well as the apparatus employed was sterilised before use. The butter thus obtained was broken by heating over a steam-bath and transferred to a separating-funnel in order to separate the milk sugar, casein and water from the pure fat. The fatty layer was shaken with freshly calcined anhydrous sodium sulphate. The moisture-free fat thus obtained was finally filtered through a water-jacketed funnel and then preserved in clean and sterilised bottles with glass-stoppers.

The milk was collected during different seasons of the year and from animals which were both stall-fed and pasture-fed.

Table No 9.

*An examination of more than forty different samples
of butterfats from cows and buffaloes has
given the following properties.*

Properties.	For Cow- butterfat	For Buffalo butterfat.
1. Physical State at 25°C ..	Varies from the oily state to thick granular and hard structure.	
2. D_{15}^C	0.9358-0.9443	0.9340-0.9444
3. Melting Point °C. ..	28.5-42.0	32.0-43.5
4. Solidifying Point °C. ...	15.0-23.5	16.0-28.0
5. Butyro-refractometric No. at 40°C	40.0-43.0	40.0-43.5

(continued).

Table No. 9.—(continued).

Properties.	For Cow-butterfat.	For Buffalo butterfat,
6. Godbole-Sadgopal Line in diffused sunlight.	Varies from colourless to violet tinge,	
7. Godbole-Sadgopal-Line in Arc-lamp-light.	Colourless ..	Colourless.
8. Iodine Value	31·5-44·95	26·5-44·0
9. Saponification Value	225·5-236·0	228·5-236·0
10. Reichert Polenske Value	0·7-1·95	0·8-2·2
11. Reichert Meissl value	21·0-34·38	24·6-35·5
12. A-Value	6·2-7·0	6·3-7·4
13. B-Value	31·0-35·52	32·9-35·5
14. Acid Value	0	0
15. Hexabromide Value	0	0
16. Sulphocyanide Value	25·5-40·8	22·65-40·0

The composition of the butterfats of cows and buffaloes as observed by an analysis of more than forty samples of each has been found to be as follows :—

Table No. 10.

Fatty Acids.	Approximate percentage in	
	Cow-butterfat.	Buffalo-butterfat.
1. Butyric Acid	4	4
2. Caproic Acid	2	2
3. Caprylic Acid	1	1
4. Capric Acid	2	2
5. Lauric Acid	4·5	4·5
6. Myristic Acid	10	9
7. Palmitic Acid	26	31
8. Stearic Acid	10	12
9. Acid higher than stearic	0	0·3
10. Oleic Acid	34·5	30
11. Linoleic Acid	5	4
12. Unsaponifiable... ..	1	0·45

CHAPTER III

The Nutritive Value and the Digestibility of Butter Fat as Compared with other oils and fats including Vegetable Ghee.

In India, one of the very important questions, often discussed is, how far Vegetable ghee or a similar hydrogenated oil can be considered suitable for nourishing the human body. One prevailing idea is that it is positively harmful to the human system; whereas, the other view is that it may not be harmful but it could not be so nourishing as butter fat. Before expressing an opinion on this vexed subject, we have put together all the information we could gather from authoritative sources.

In drawing inferences from the physiological observations and experiences of specialists on the subject in Europe and America, one important aspect has got to be borne in mind and that is, that both in Europe and in America, as previously referred to, Butter-fat as such does not play a very important part in the daily diet. The competition in those countries is between pure Butter on the one hand and commercial Margarine on the other. For the daily use on the breakfast table, what is required is either butter or margarine, both of which, on account of their semi-solid consistency, have got the property of being spread on the bread. The rich and the poor alike have the same type of the menu, the difference being one of quality. The poor cannot afford to pay for the expensive butter. Margarine *i.e.*, an emulsion of oils and fats with water and

a flavour of butter takes its place. In America particularly, a very interesting and serious controversy is going on between the two schools of margarine *vs.* butter. A certain amount of data is available,¹ but on the whole it appears to be more of a propagandist type than of a scientific nature. The number of factories manufacturing margarine and the sums of capital locked up in their working are so large that the margarine school is naturally endeavouring to hold its own against the established popularity of the butter school. The researches of the margarine school regarding the physiological effect of margarine in the human system have got to be carefully interpreted before they can be made applicable to the oils and fats as used in India in their dehydrated form. The popularity of natural butter as compared with that of margarine and the preference given to its use even in the largest margarine manufacturing countries—in the face of a regular and enormous propaganda made in favour of the latter—is in itself a strong argument in favour of the usefulness of butter.

The views of the margarine school can be briefly summarised as follows: “According to the best scientific information, a pound of one kind of fat yields exactly the same amount of heat and muscular energy as a pound of any other kind of fat, and one kind of fat is digested with practically the same ease and completeness as any other kind. On the basis of actual utility of a fat as a food, there is, therefore, no choice except in the matter of cleanliness, soundness, freedom from contamination, price, convenience in handling, suitability for certain cooking

(1) The literature can be had of the “Institute of margarine manufacturers, 1049, Munsey Buildings, Washington, D. C.” We have to thank them for a number of useful pamphlets to which we have referred in our Bibliography.

purposes, and last, but by no means least, palatability".¹ We should like to once more emphasize here that the above statement refers to margarine which is not identical with vegetable ghee and other dehydrated fats. The inference, therefore, cannot necessarily be the same.

As against this, the German school of chemists and physiologists have given us useful data in the form of their experiments on the dehydrated oils and fats. These researches interest us because they throw proper light on the immediate problem before us. According to Koenig, the oils and fats which are easily decomposable are also easily absorbed by the human system. Luehrig² tried to ascertain the relation between the relative velocities of saponification of oils and fats and their relative digestibility, *i. e.*, to say the corresponding absorbability by the system. He found that by taking butter, margarine, lard, cotton-seed oil, sesame oil, etc., there was no appreciable difference in the velocity of saponification of these fats. This argument, of drawing an inference regarding the digestibility from the saponification velocities is open to one serious objection and that is that in estimating saponification values, half normal alcoholic solutions of caustic potash are used which are undoubtedly very strong alkalies which, we think, cannot be compared with the small (though active) quantities of ferments in the human system. This analogy, therefore, does not hold good. It has also been proved that the fats with higher melting points are less digestible than those with lower melting points.³ Taking, even, the free fatty acids by themselves (which are invariably present in all Indian oils and fats, since the process of chemical refining is hardly

(1) Abbot, J. S : The composition and food value of margarine, p. 10.

(2) Chem Ztg. 24, 647, (1900).

(3) Zuntz, Bahrung und Ernährung 1918.

done), it is found that the higher fatty acids like palmitic and stearic acids pass off through the human system unused and unabsorbed, whereas free oleic acid is taken up and absorbed by the system and by its mere presence also helps partly to absorb the otherwise indigestible acids like palmitic and stearic.¹

Accordingly, it is found that oils and fats whose melting points lie below the normal temperature of the body (about 36° centigrade) are absorbed and digested in the intestines almost upto 97 to 98 per cent., whereas in the case of tri-stearin (having a melting point 71° C and which is, by the way, the major constituent of animal fats and some hydrogenated oils), it is found that only 9 to 14 per cent. is used, that is to say, the remaining 91 to 86 per cent of the tri-stearin only helps in loading the system without in any way benefiting it.² The assimilability of free acids is, in general, a function of its molecular weight, *i.e.*, the lower ones are more easily absorbed than the higher ones; although in the case of unsaturated acids like oleic they are more easily absorbed than similar but saturated acids both having the same number of carbon atoms³ not merely because of their low melting points but also because of their unsaturated character which is responsible for their greater chemical activity. This appears to our mind to be a highly scientific reasoning because this relation of a uniform change of chemical and physical properties with an increase in the molecular weight fits in with a long established theory of chemical constitution. From the above mentioned reference, it is clear that from the point of view

(1) E. Rost, *a. a. o.*

(2) Arnschink, *Zt. f. Biol.* 26, 434 (1890).

(3) Spieckermann, *Zt. Nahr. u. Genu* 27, 83, (1914); Rudolf, *Zt. Physiol Chem.* 101, 99, (1918).

of the chemical composition of butter fat, vegetable ghee, coconut oil, sesame oil, etc., so far as digestibility and absorbability by the system are concerned, we place these in the following order, (1) butter fat, (2) coconut oil, (3) other oils rich in oleic glycerides such as sesame, and safflower oils, (4) animal fats and hydrogenated oils of vegetable origin (vegetable ghee) or of animal origin. The oils and fats which are hydrogenated in such a manner that their melting points lie between 36-40°C are more easily absorbed than those hydrogenated fats which possess higher melting points.¹ Further it has been observed that water soluble free fatty acids starting from C₄ upwards and also their corresponding aldehydes are poisonous in their character. It is, therefore, that rancid fats are likely to be injurious to the health of human beings.

As regards the supply of vitamins, butter fat is perhaps the best source of vitamin A.² The other fats are very deficient in this respect. In addition to vitamin A, vitamin D is also present in butter fat. There is a strong reason to believe that the source of vitamins in butter fat is the green grass and leaves on which the cattle feed. There can be, therefore, no two opinions on the point that in the matter of digestibility and vitamin potency, butter fat occupies the most privileged position of being the best of all the oils and fats for human use. It might be noted in this connection that every province in India is partial to a particular oil depending upon its availability and its peculiar flavour. Tastes cannot and should not be discussed.

We are further of opinion that chemists and physiologists in India should join hands and try similar

(1) S. Ueno, M. Yamashita u. Y. Ota : Jowin. Soci. Chem. Ind. Jap. Suppl. 1927, 105.

(2) Sherman, H. C. and Smith, S. L, Vitamins, pp. 184, 185, 209.

experiments on the digestibility and assimilability of Indian oils and fats under Indian conditions, as the inferences drawn from experiments performed in Western conditions can only be accepted with reservations, although there can be no denying of the correctness of the purely scientific results.

CHAPTER IV.

The comparative merits of Cow-butterfat and Buffalo-butterfat as human Food-materials.

A very important question has been raised regarding the comparative merits of butterfats obtained from (a) Cows and (b) Buffaloes with a view to find out which of the two animals deserves greater attention and care from a rural point of view in India. To answer this question, it is necessary to carefully examine the whole problem both from the chemical and physiological points of view. In our book on Butterfat, (N. N. Godbole and Sadgopal, *first edition* 1930, pp. 10-13) we have explained that ghee as obtained from cows and buffaloes is a better nourishing material than tallow or lard or vegetable ghee and in doing so, we have quoted European experimenters in original. The point before us is one of a further discussion on the comparative merits of the ghees obtained from cows and buffaloes. It should be borne in mind that what is sold in the Indian market under the name of butter or ghee may be either cow-butter or buffalo-butter or a mixture of both. Ordinarily, no distinction is made between the two even in the matter of cost. Unless specially demanded for medicinal purposes, no special distinction is made between the two varieties of butter or butterfat. However, it is useful to examine the whole problem from a scientific point of view.

Although the cow and buffalo are two distinct species of animals, yet so far as the problem of their butterfat is

concerned, the following facts are to be borne in mind. Both the animals can be looked upon as Nature's Butter-making machines, although taken as a class, the buffalo is economically more valuable than the cow for dairy purposes. The buffalo is hardly known in the cold countries and therefore, so far as general literature goes, in Europe, very little interest is taken in the buffalo butterfat. All the same, the cow is so well-fed (on oil-cakes) in the well-to-do-European countries that the composition of its butterfat often equals in composition the butterfat obtained from buffaloes in poor India. Even in India, neither cow's milk (and its derivative-Ghee) nor buffalo's milk (and its derivative-Ghee) are constant in their composition. This variation is due to the race, the breed of the animal and the seasonal differences in the food. Therefore, the animal remaining the same, the composition of its milk and butter will vary from season to season and from province to province. A cow well fed in the Berar or the Punjab gives milk and butter which almost beat in composition the milk and butter obtained from buffaloes in Madras or East Bengal. The one great difference between the cow in India and the cow in Europe is the great resistance which the Indian cow offers to all bacterial diseases like T. B., dysentery, typhoid and cholera etc., because of the blessings of nature. Practically all through the year, the Indian cow remains outdoor and enjoys the warmth of the sun. It is for this reason that in European countries, the dairy cow is supposed to be responsible for spreading some of the above mentioned diseases through its milk. Thanks to the ample supply of air, light and warmth, the Indian cow is far superior (in resisting the germs of certain diseases) to the European cow. It has also been shown that the buffalo is still more resistant in this respect than the cow.

It should be clear from the above that there is not much of a serious qualitative difference between the composition of the two butterfats although a certain amount of quantitative difference exists as will be presently explained with the help of the following tables. In order to show the superiority of the butterfat of cows and buffaloes over other fats commonly used in Europe, we are giving comparative tables for tallow and lard etc. We are also including coconut oil, because along the west coast of India, it is used as a substitute for ghee.

Table No. II.

A comparison of the Melting and Solidifying Points of various fats.

Property	European Cow's butterfat (according to Holde, Bleyberg and Grun)	Buffalo's Butterfat (according to Grun)	Indian Butterfat from average of about fifty samples each. (according to Godbole & Sadgopal)	
			Cows	Buffaloes
Melting Pt.	28-41°C.	31-38°C.	28.5-42°C.	32-43.5°C
Solidifying Pt.	15-25°C.	24.5-29°C.	15-23.5°C	16-28°C.

No.	Fats.	Melting Points °C. (according to Holde-Bleyberg)	Solidifying Points °C.
1.	Mutton Tallow	44-55	32-45
2.	Beef Tallow	40-50	30-38
3.	Lard	28-46	22-32
4.	Coconut	20-28	14-25

Before we examine the detailed composition and the constituents of various fats, it is necessary to understand

that both the oils and fats, whether of animal origin or vegetables origin, are mixtures of mixed glycerides of acids of low and high molecular weights. In these again, the glycerides of high molecular weights are of either saturated acids or of unsaturated acids. Whenever the oils and fats are introduced into the human system, their assimilability and digestibility are dependent upon the nature of the acids. The glycerides of saturated acids of high molecular weights are not digestible and, therefore, load the human system and pass off undigested and unassimilated. They are likely to do, therefore, some harm to the system and do no good whatsoever. This is mainly due to the fact that the glycerides of saturated acids of high molecular weights have melting points higher than that of the body temperature itself. According to Lewkowitsch, the melting points of the saturated glycerides of high molecular weights vary between 56.5-75°C. Therefore, substances like mutton and beef tallows are the least useful if not also the most undesirable for feeding the human system. The liquid glycerides of the unsaturated acids of high molecular weights, particularly those of oleic and linoleic acids, on the contrary, are not harmful as those of the corresponding stearic-glycerides of about the same molecular weights. These are easily absorbed and are known to be beneficial. The glycerides of saturated acids of low molecular weights ranging from those of butyric to those of myristic acids are known to be easily assimilated by the system because of their very low melting points and easy digestibility and assimilability. The remarks made above for the non-assimilability of the solid glycerides of the saturated acids of high molecular weights are modified to a certain extent when the glycerides of either unsaturated acids or acids with low molecular weights are mixed with them. The

proportionate percentage of the glycerides of saturated acids of high molecular weights on the one side and that of the glycerides of either unsaturated acids or acids of low molecular weights on the other decides the comparative assimilability and digestibility of any given oil or fat. The presence of free fatty acids in general in any given oil or fat, either arising out of the natural causes or as a product of rancidity is always harmful to the human system. That is why in Europe every edible oil or fat is refined for removal of the free acids before it is sold in the market, or before it is used for the manufacture of margarine. The following table, it is hoped, will throw light on the influence of the composition on the digestibility and assimilability of the fats taken above for comparison.

Table No. 12.

A comparison of non-assimilable constituents of various fats.

No.	Constituents.	In Cow-Butterfat according to Holde, Bleyberg and Grun.	According to Godbole-Sadgopal in	
			Cow-Butterfat (average of about forty samples In each case.)	Buffalo-butterfat.
1.	Stearic Acid.	1.1-5.9%	10%	12%
2.	Palmitic ..	11.8-17.5%	26%	31%
3.	Acids higher than Stearic.	0.3%

Table No. 12—(continued).

No.	Fats	Percentage of	
		Stearic Acid.	Palmitic Acid.
(According to Holde-Bleyberg.)			
1.	Mutton Tallow.	25-35.5%	24-27%
2.	Beef Tallow.	24.5%	27-29%
3.	Lard.	7.8-15%	24.6-32.2%
4.	Coconut oil.	0.8-5.0%	4.3-7.5%

Table No. 13.

A comparison of assimilable constituents of various fats

No	Constituents.	Percentage in Cow-butterfat according to Holde-Bleyberg and Grun.	Percentage according to Godbole-Sadgopal in	
			Cow-butterfat (average of about forty samples)	Buffalo-butterfat
1.	Oleic Acid	24.47%	34.5%	30%
2.	Linoleic Acid	..	5.0%	4%

No.	Fats	Percentage of	
		Oleic Acid.	Linoleic Acid.
(According to Holde-Bleyberg)			
1.	Mutton Tallow.	36.43	2.7-4.3
2.	Beef Tallow.	43.44	2.6
3.	Lard.	50.4	10
4.	Coconnt oil.	10-10.2	1

Table No. 14.

*A comparison of easily-assimilable constituents
of various fats.*

No.	Constituents.	Percentage in Cow-butterfat according to Holde-Bleyberg and Grun.	Percentage according to Godbole-Sadgopal in	
			Cow-butterfat	Buffalo-butterfat
(average of about 40 samples).				
1.	Butyric Acid	2.9-4.5	4	4
2.	Caproic Acid	1.3-2.4	2	2
3.	Caprylic Acid	1-1.9	0.9	0.9
4.	Capric Acid	1-1.5	2	2
5.	Lauric Acid	3.6-6.4	4.5	4
6.	Myristic Acid	10.4-20.1	10	9

No.	Fats	Percentages of acids of					
		Butyric	Caproic	Caprylic	Capric	Lauric	Myristic
(According to Holde, Bleyberg and Grun)							
1.	Mutton Tallow	2.4.6
2.	Beef Tallow	2.2.5
3.	Lard
4.	Coconut oil	..	0.2-2.0	6-9.5	4.5-10.7	45-51	16.5-20

Note : In the chemistry of oils and fats, this total content of the above mentioned fatty acids of low molecular weights (the acids being steam volatile and water soluble or water insoluble) is represented by what is known as Reichert-Meissl (R.M.V.) and Reichert-

Polenske (R.P.V.) values. The content of Butyric acid whose glyceride is present only in butterfat, is empirically represented as B value.

In this connection, we would like to point out our difference of opinion from that of Prof. Rao Bahadur Sahasrabudhe of Poona according to whom "the percentage of volatile and soluble acids is greater in cow-ghee and consequently it is more easily digested." The results obtained by us from an analysis of not less than forty samples of each show that this part of steam-volatile and water-soluble and insoluble acids does not vary appreciably in the ghees from the two animals, so as to make a difference in their digestibility. Again even the R. M., R. P., A and B values also do not differ very much. On the other hand, the larger percentage of palmitic and stearic glycerides present in the case of buffalo butterfat makes it comparatively less digestible and unassimilable than cow's butterfat.

In connection with the above tables, the following facts should also be noted before drawing any final conclusion :

(I) The partiality shown by certain people to particular oils and fats can also be accounted for, by the difference in matters of taste and flavour with every individual. In India, in certain parts, coconut oil is used as an edible oil whereas a very common use of sesame and safflower oils etc., is noticed else-where. It is to be remembered that the oils like sesame etc., are also easily digestible being very rich in oleic-glycerides.

(II) Nutritive value of fats cannot be exclusively based upon their relative heats of combustion. Although fats may be replaced isodynamically by equivalent quantities of other fats or even carbohydrates, the deficiency in vitamin

potency plays a very important role in the building up of the healthy tissues in the human body.

A summary of the above tables is given as follows :

Table No. 15.

No.	Fats	Non-assimilable Palmitic + Stearic glycerides.	Assimilables Oleic + Linoleic glycerides.	+	Easily Assimilables Lower fatty acid glyceride.
1.	Beef Tallow	52%	46%	+	2%
2.	Mutton Tallow	55%	42%	+	3%
3.	Lard	40%	60%	+	—
4.	Buffalo-butterfat	44%	34%	+	22%
5.	Cow-butterfat	36%	40%	+	23.5%
6.	Coconut Oil	9%	11%	+	80%

It is very interesting to note that human fat which is a synthetic assimilative product built up out of the digested part of the oils and fats taken in the form of food is known to possess the following composition and has a melting point between 15-22°C, Saponification value 190—200, acid value less than 1, Iodine value 59—73, R. M. value 0.3—0.6 :

Composition of Human Fat.

Stearic Acid	4%
Palmitic Acid	16%
Oleic Acid	66-87%
Cholesterin	0.18%
Lecithin	0.8%

It seems probable, therefore, that it is the lower and unsaturated glycerides which are primarily responsible for building up human fat. In the case of newly born children, the oleic acid amounts to only 43 %.

We fail to appreciate the remarks of Mr. Bruen, the livestock-expert to the Government of Bombay, who says "Buffalo milk is hard to digest by a person of any age, because the excess of fat in buffalo milk when it forms soap in the intestine is hard to digest with the usual amounts of salt, and it takes the deficient quantity of the mineral salts from bones which are consequently weakened." This seems to us to be a very peculiar explanation as the main constituent of the bones is Calcium Phosphate, a substance which is not only insoluble in water but cannot react chemically either with the soap or the products of hydrolysis formed during the process of the digestion of the fat. We find absolutely no scientific reason to condemn the buffalo milkfat as a nutritive fat for human use.

Our conclusions based on all the material at our disposal are as follows :—

1. Cow's butterfat is known to have iodine in its composition whereas no data is available on this point in the case of buffalo butterfat.

2. Both cow's butterfat and buffalo-butterfat contain vitamins A and D but cow's butterfat is richer in the vitamin A while the other is richer in vitamin D.

3. Butterfat as such is any time better than tallow, lard or vegetable ghee.

4. Cow's butterfat is richer than the buffalo-butter-fat in the total assimilable and digestible part and is, therefore, comparatively more suitable for children and weaklings.

5. From an economic point of view, the buffalo is a better butter-producing machine than the cow.

We are of opinion that it is worth while trying experiments in India on oils like sesame and coconut etc., which

are rich in assimilables but poor in vitamins regarding assimilability.

Of the two butterfats, cow's is nearer to the composition of human body-fat than the buffalo-butterfat.

Some additional data.

Recently, physiological experiments have shown that the following quantities of various foodstuffs are individually capable of yielding 3600 calories of heat-energy to the human system :

Butter	1 lb.
Bread	3 lbs.
Meat	3.5 lbs.
Potatoes	9.5 lbs.
Bananas	12.25 lbs.
Oranges	21.25 lbs.
Eggs	53

Further, in experiments on guinea-pigs, it has been found that while the proteins of wheat are 26.7% digestible, the proteins of milk are 66.2% digestible. One pound of fresh cheese supplies about as much digestible proteins as four pounds of wheat.

Regarding the supply of mineral matter, it is to be noted that one gramme of cheese supplies as much calcium as 45 grammes of wheat flour or 95 grammes of polished rice. In fact, it has been experimentally proved that a pound of milk contains more calcium than a pound of lime-water.

From the point of view of vitamin-potency, it has been further shown that one gramme of butter supplies as much vitamin A as 75 grammes of vegetable oils and that one gramme of cream contains as much vitamins as one gramme of cod-liver oil.

The comparative food-values enumerated above clearly establish the superiority of pure butter over other foodstuffs.

From the statistics available in India, it appears that there are many more lepers in the southern parts of India than in the northern. One of the explanations offered by medical people for this fact is that less milk is taken daily per head in the southern parts than in the northern and as a consequence, it is inferred that milk is a preventive to a disease like leprosy.

Another very interesting fact has been very recently made known in Europe that successful injections of milk have been given to prevent gonorrhoea.

CHAPTER V

Adulterants—their constants, composition and tests.

The following is the list of the animal and vegetable oils and fats usually employed for adulterating Butterfat :—

(a) *Animal Fats* :—Lard and Tallow,

(b) *Vegetable Oils & Fats* :—Coconut oil, Cotton-seed oil, Cotton-seed Stearine, Sesame oil, Arachis oil, Soyabean oil, Mohuwa oil, Poppy-seed oil, Safflower oil, Illippe butter and Poli oil.¹

(c) *Hydrogenated Products* :—In the form of Vegetable Ghees.

N. B.—During the last few years, five or six factories have been established in India for manufacturing hydrogenated oils (vegetable ghee!) using largely ground nut oil and cotton seed oil according to the market prices. Imported hydrogenated fish oil from Japan, at one time, played a havoc in the Bombay market in making cheap imitation butter. A study of the correct composition of these Indian-made Vegetable Ghees should be made to ascertain their food value and assimilability.

(1) Barnes and Singh. *Analyst*, 1916, 242,72,

Table No. 16.

General characteristics and composition of various fatty adulterants of butterfat.

Number	Name.	Saponification Value	Iodine Value.	Sulphocyanide Value.	Reichert Meissl value.	Acetyl Value.	General composition.
1	Arachis oil ..	180—197	86—98	70—78 74.0*	0.4—1.6	3.4—9	Palmitic acid 4—8.5%, stearic acid 4.5—6.5%, Oleic acid 51.6—80%, Linoleic acid 7.4—26%, Arachic acid 4—5%, Caproic acid 2—2.0%, Caprylic acid 6—9.5%, Capric acid 4.5—10.7%, Lauric acid 45—51%, Myristic acid 16.5—20%, Palmitic acid 4.3—7.5%, Stearic acid 0.8—5%, Oleic acid 2—10.2%, Linoleic acid 1%.
2	Coconut oil ..	246—268	7—10	6—10 10.9*	0—8.5 (Reichert Polensky value 16—20.5)	1—12	Oleic acid 30—30.5%, Linoleic acid 40—45%, Higher Fatty acids 20—23%. Water Soluble (Butyric and Isovaleric) acids 7—8%.
3	Cotton-seed oil ...	191—198	101—120	61—65	0.2—1	7.6—21	Palmitic acid 23—25%, Stearic acid 31—34.5%, Oleic acid 39—43%, Linoleic acid 2%.
4	Dolphin Fish oil ..	212—290	24.5—133	..	39—112	..	Palmitic acid 32.2%, stearic acid 7.8% Oleic acid 60%, Myristic+Palmitic+Stearic acids 8—10%, Oleic acid 5—20%, Linoleic acid 22—59% Linolenic acid 21—45%.
5	Kakao Butter ..	192—202	34—38	32—35	0.1—0.4	..	Palmitic+Stearic acids 34—6%. Oleic -63—66%.
6	Lard ...	193—200	46—66	44— 47.25*	0.3—0.9	2—10 (Rancid)	Palmitic+Stearic acids 7%. Oleic acid 28%, Linoleic acid 59%, Oxyacids 0.8%.
7	Linseed oil ..	187—197	170—180	112—119	0.9	8	Oleic acid+Linoleic+Linolenic acids 72%, Palmitic and higher fatty acids 11.8%.
8	Mowra Fat ..	187—195	53—68	48 48.6*	0.2—1.7	..	Stearic acid 4.6%, Arachic acid 0.4% Oleic acid 48.1%, Linoleic 36.8%.
9	Poppyseed ...	189—198	131—143	78—79	..	13	Myristic acid 2—2.5%, Palmitic acid 27.29% Stearic acid 24.5%, Oleic acid 43—44%, Linoleic acid 2.6%.
10	Safflower oil ..	174—194	138—150	..	0.2—1.6	13—16	Myristic acid 2—4.6% Palmitic acid 24—27%, Stearic acid 25—30.5%, Oleic acid 36—43, Linoleic acid?.
11	Sesame oil ...	186—195	103—112	75—77	0.1—0.4	10—11	
12	Tallow—(Beef) ...	190—200	32—47	39.4 34.9*	0.1—0.6	2—9 (Rancid)	
13	Tallow—(Mutton) ...	192—198	31—47	35.0*	0.1—1.2	..	

NOTE:—The above chart has been prepared from the tables given on pages 786—809 of *Kohlenwasserstoffole und Fette* by Holde-Bleyberg, Ed. 1933.

The sulphocyanide values marked with asterisk (*) are taken from a paper published by N. N. Godbole and Sadgopal; *Allgemeine Oel-u-Fett-Zeitung*, 1934, Nov. pp. 435—438.

Detection of Hydrogenated Fat (Test for Nickel).

Hydrogenated fats as such, are often recognized by their characteristic smell (flowery), but it is difficult to recognize them in the presence of fats having the same consistency (*eg.* tallow). The following tests are conclusive if very positive results are obtained, since, in many hydrogenated fats, the traces of nickel are completely removed, and iso-oleic acid is totally absent.

Procedure :—50 to 100 gms. of fat are warmed over a water-bath for half an hour with an equal volume of conc. HCl, with frequent shaking. It is then filtered through a moist filter paper into a porcelain dish, evaporated to dryness and the residue extracted with a small quantity of HCl. The solution is then made strongly alkaline with ammonia, and then a pinch of lead peroxide, some drops of NaOH solution and 8-10 cc. of a 1% solution of dimethylglyoxime are added to it. The solution is heated to boiling and filtered.

Conclusion :—In case a hydrogenated fat is present, the filtrate is coloured red, the intensity of the colour depending upon the amount of nickel present. A weak yellow colour may be formed by the presence of organic decomposition products.

ISO-OLEIC TEST.

Explanation :—A comparatively high content of solid unsaturated fatty acids especially of the isomers of unsaturated fatty acids, and therefore, a comparatively higher iodine value, of the fatty acid separated according to the lead-salt-alcohol method, are characteristics of hydrogenated fats.

Procedure :—After the separation of the unsaponifiables, the total fatty acids are taken as the starting material. 2-3 gms. of the fatty acids are dissolved in hot

alcohol, and a solution of about 1.5 gms, lead acetate in alcohol is added hot. The mixture (the volume of which is about 100 cc.) is allowed to cool gradually, and kept over night. The clear supernatant liquid over the lead-soaps should give a distinct pptte. with sulphuric acid, otherwise more of lead acetate solution is added. The pptte. is filtered by suction and washed with cold alcohol, until the filtrate is clear. The pptte. is washed out into a beaker with 100 cc. of alcohol, 0.5 cc. of glacial acetic acid is added, the whole is heated to boiling and is then allowed to cool to 15°C. The mixture is again filtered and washed.

The lead-soaps are then brought into a beaker and extracted with ether. The solid fatty acids are separated from the insoluble lead-soaps by means of dilute nitric acid and removed as an ether-extract. The iodine value of the extracted fatty acid is determined.

Conclusion :—The iodine value of such fatty acids from natural fats is generally from one to two (1 to 2), with tallow upto 5, but the iodine value of those from hydrogenated fats having a consistency of from lard to tallow, is above 20 and sometimes even upto 50 or over.

Sesame Oil Detection.

There are two methods of sesame oil detection, Soltsien's test and Baudouin's test. Soltsien's test is more reliable than the Sesamol or Baudouin's test. The latter, is however, almost exclusively employed, because as Baudouin's test, it is described under the official methods, and also because it is a colour-reaction and is easy to carry out in commercial analysis.

Soltsien's Test

Procedure :—The oil or melted fat under examination, benzine (b. p. 70-80°C), and fresh Bettendorf's Reagent (5 pts. solid stannous chloride, and 3 pts. fuming-hydro-

chloric acid) are thoroughly mixed in the porportion 1 : 2 : 1 in a test tube, and the whole is dipped in water at 40°C. After the separation of the stannous chlride solution, the test tube is dipped in water at 80°C. so that the benzine layer evaporates off without boiling.

Conclusion :—Red colouration of the lower layer shows the presence of sesame oil.

The reaction is reliable even when the Sesamol or Baudouin's test fails, for example, in the case, of rancid fats and certain olive oils which give a positive Sesamol test as also in the case of tar colours.

Sesamol or Baudouin's test.

*Procedure :—*6 gms. of fat are dissolved in 5 cc. petrol ether and shaken for half a minute with 0.1 cc. of a fresh 10% alcoholic furfural solution, and 5 cc. conc. HCl (sp. gr. 1.19).

If the oil turns red by shaking with HCl (sp. gr. 1.125). then it should be examined according to the Soltsien's test.

*Conclusion :—*Above 1% sesame oil can be detected by the red colouration of the acid layer. Sometimes even 0.5% sesame oil is recognisable by a rosy colour of the said layer. In the absence of sesame oil, a yellow or at most a brown-yellow colour is formed.

Cotton-seed oil test.

*Procedure :—*About 2 cc. of the oil are heated under a reflux condenser in a bath at 115°C. with 2 cc. of a 1% sulphur solution in a mixture of carbon disulphide and pyridene (1 :1).

*Conclusion :—*If more than 1% of cotton seed oil is present then the mixture becomes red within a short time. If no colouration is observed within 5 minutes, a further

addition of the sulphur solution is made. If after another 5 minutes no red colouration appears, then cotton-seed oil is absent.

In general, oils which contain cotton-seed oil and are strongly heated or bleached, do not give the reaction, or at most give it to a slight extent. On the other hand, fat from animals fed on cotton-seed cake give the reaction. In such cases, the phytosteryl acetate test is decisive.

Note :—Kapok and Baobab oils always give this reaction; however, 5 cc. of their fatty acids (melted and dried) when shaken in cold with 5 cc. of 1% solution of silver nitrate in absolute alcohol, give an intense brown colouration, while the fatty acids from cotton-seed oil only slightly reduce the silver nitrate solution.

Detection of vegetable fat by the phytosteryl-acetate test.

(Digitonide-method.)

Explanation :—Phytosterol, Cholesterol and a few other sterols form additive compounds (digitonides) with digitonin and they can be separated from the fats by the method mentioned below. Steryl acetate and sterols prepared from digitonides can be qualitatively differentiated in such a way that the presence of phytosterol and hence that of vegetable fat can be recognized. By the negative result of the test it should not be concluded that animal fat is necessarily present.

Procedure :—10 to 50 gms. of fat, according to the expected yield of sterols, is saponified in a 500 cc. flask with 100 cc. of alcoholic KOH solution (200 gms. of pure KOH dissolved in 1000 cc. of 75% alcohol). The flask should be covered with a watch glass and the fat saponi-

fied over a boiling water-bath for half an hour. The soap solution is first diluted with an equal volume of hot water and decomposed with 50 c. c. of 25% HCl. The separated fatty acids are filtered through a moist thick filter paper in a hot filter-funnel, and when all the water layer is run through, the fatty acids are filtered through a dry filter-paper in a 200 c.c. beaker. In order to prevent the turbidity of the liquid passing through the filter-paper, the latter is first filled upto half with hot water and then only the liquid with the fatty acid is poured over it.

The total quantity of fatty acids, or in case of high sterol contents correspondingly lesser quantity is treated at 60-70° Centigrade with 20-50 c. c. of 1% digitonin solution (0.2 gms. of digitonin dissolved in 25 c. c. of 96% alcohol; the digitonin is to be tested for its efficiency before hand with a mixture of 48 gms. lard and 2 gms. cotton-seed oil). After heating to 70°C for an hour with frequent stirring, 20-25 c. c. of chloroform is added to it and immediately filtered through suction filter. If no precipitate appears within an hour, the result of the sterol test is considered to be negative.

The digitonide crystals are washed with warm chloroform-ether mixture till free from fatty acids. The filtrate should no longer give any pptte. with digitonin solution. The residue on the filter paper, after drying at 100°C for 10 minutes is treated with ether to remove the last traces of fatty acids and filtered. A portion of the pure digitonide is shaken with 3-5 cc. of pure acetic anhydride for 10 minutes and, then to it while still hot, is added four times its volume of 50% alcohol. After cooling for 15 minutes in cold water, the separated sterylacetate is filtered by suction and washed with 50% alcohol. The solution of the acetate in a little ether is again evaporated to dryness and the residue recrystallized 3-4 times from

one cc. of alcohol. The fractions are separated by pressing between porous earthen ware plates and the melting point of the thrice crystallised fraction is determined in a capillary tube.

Conclusion :—Cholesteryl acetate melts at 114.3°C . (corrected) and phytosteryl acetate at least 10° higher. If the last crystallised fraction melts at 117°C . (corrected) or higher, phytosterol is considered to be present and hence the presence of a vegetable fat is inferred.

In presence of wool fat as also in the case of oxidised (blown) oils and fats and those hydrolysed above 200°C ., the test fails.

Sterols are obtained on prolonged boiling of the digitonides in Xylol and extracting with ether. The separated sterols are dissolved in 5-25 cc. of absolute alcohol and allowed to crystallise for a long time in a covered dish. Cholesterol melts at 148.4°C . (corrected) and Phytosterol melts at 132 to 144°C . (corrected).

For the microscopical examination a drop of the alcoholic solution of sterol or a crystal is placed on the slide and its crystalline structure studied. Presence of Cholesterol is shown by broad rhombic plates while thin pointed needles show the presence of Phytosterol. Mixed crystals are predominantly shaped like those of Phytosterol.

CHAPTER VI

Discussion of the Methods of Qualitative and Quantitative Analysis employed hitherto.

The adulteration of Butterfat with vegetable oils and animal fats has attained a very great importance in this country during the last score of years. Latterly, in the local vegetable Ghee, the sophisticator has had a handy substance which possesses all the external physical properties of Butterfat. This adulteration is freely practised because of the complexity of the chemical composition of the Butterfat and the difficulties it presents in its analysis and detection. With the growth of scientific knowledge, the adulteration is being carried on so systematically and intelligently that its detection also is now developing into a special branch of analytical science. The aim of writing this chapter is to carefully examine the current methods for detecting qualitative and quantitative adulteration in Butterfat and then to present the most suitable solution of the problem.

Before attempting to define the most suitable methods for the said purpose, it is necessary to find out how far the methods already suggested are applicable in this case.

1. *Saponification Value*:—The mean saponification value of butterfat may be taken as 227, according to Kottstorfer¹, the maximum and minimum known being

(1) Lewkowitsch J. Chem. Tech. and Ana. of Oils, Fats and Waxes, Ed. 6, Vol. 2. p. 381.

260 and 225. This value alone cannot be relied upon as a test of purity of butterfat. It is very easy to prepare suitable mixtures with vegetable ghee, tallow and coconut oil in such a manner that the adulterated sample of butterfat can have a saponification value equivalent to the average value of butterfat. For example, a mixture of the following :—

50 parts of pure butterfat, (Saponification value 227).

25 parts of tallow, (Saponification value 200)¹, and

25 parts of coconut oil, (Saponification value 255)².

will give a product having the same saponification value as that of a pure sample of butterfat. The determination of the saponification value alone cannot by itself, be relied upon to ascertain the purity of butterfat. It can be utilized as a supplementary test to corroborate information obtained by other tests.

2. *Specific Gravity*:—Sp. gr. number alone is of limited value in estimating the purity of a sample of butterfat. Below is given a table giving the specific gravities of a number of oils and fats :—

Specific Gravity at

	100/15.5°C	15.5°/15.5°C.
Butter-fat	.. 0.863	0.938
Lard 0.860	0.936
Tallow 0.860	0.947
Cotton-seed Oil	.. 0.872	0.923
Sesame Oil	.. 0.867	0.923
Arachis Oil	.. 0.863	0.917
Coconut Oil	.. 0.874	0.926

(1) Lewkowitsch J. Chem. Tech. and Anal. of oils, fats and waxes, ed. 6, vol. 2, p. 768 ;

(2) Ibid. p. 645.

It would be seen that small quantities of vegetable oils and animal fats are entirely undetectable and an incorporation of a judiciously prepared mixture of butterfat with coconut oil and animal fats will go without being detected by an abnormal specific gravity number. This test only helps in those cases where adulteration exceeds 30 per cent.¹ It is enough to point out in this connection that lard, beef and mutton tallows, tung oil and butterfat have got very nearly the same sp. gr.² Recently a co-called "Ghee tester" based on this principle of determining the sp. gr. at the temperature of 80°C. has been suggested.³ The general rule is that the glycerides of soluble acids increase the sp. gr.⁴ It must also be noted that the sp. gr. of oils and fats containing free fatty acids is lowered with the increase in the amount of free fatty acids.⁵ Therefore, merely by taking a sp. gr. reading (which also varies with the temperature) it is quite unsafe and unreliable to pronounce any opinion on the purity of butterfat. According to Elsdon⁶ also "It is quite easy to adulterate butterfat in such a way that no indication of the fat would be given by the sp. gr."

3. *Melting and Solidifying Points* :—An examination of not less than 200 samples of pure ghee from various parts of India has shown that the melting point of this fat lies between 28.5-43.5°C. Therefore, for purposes of detecting adulteration in ghee, this test is hardly of any analytical value. This can, however, be employed

(1) Lewkowitsch, J. Chemical Technology and Analysis of Oils, Fats and Waxes, ed, 5, Vol. 2. p. 853 and Vol. 1, p. 311 ;

(2) Ibid. Vol. 2. p. 768 and Vol. 1, p. 331 ;

(3) Nandlal, D. Gulla, "Ghee-Tester" ;

(4) Lewkowitsch, J. Chem. Tech. and Ana. of oils, fats and waxes, Vol. 1, p. 312.

(5) Ransome, A, O. Journal of the Society of Chemical Ind. 1912, 672 ;

(6) Elsdon : Edible Oils and Fats, p. 384 ;

to confirm when the adulterant is known to be a fat of a high melting point. The melting point, it should be noted, is relied upon only in the case of pure and simple substances having a sharp melting point. Polenske¹ proposed, with the help of "difference numbers between melting and solidifying points" to detect the admixture of one animal fat with another. Boamer and Limprich² pointed out that the method was useless for the detection of foreign animal fats in butter fat. Even the melting point of cholesteryl-acetate isolated from butterfat which rarely exceeds 114°C³ after a large number of crystallizations, has been found to be raised by feeding the animal on oil cakes.⁴

4. *Microscopic Examination*:—J. Brown,⁵ as early as 1874, has suggested the use of a Polarisation Microscope for the testing of butter-fat. The method was tried by the present authors and was found to show no characteristic behaviour in the case of oils and fats. This method, therefore, is not of any great value.

5. *Spectroscopic Examination*:—An attempt was made to use a spectroscope to find out whether oils and fats give any special absorption bands. Only in the case of mustard oil could any absorption be observed. In the case of ground nut, coconut and almond oils and so also in the case of butterfat, nothing characteristic was found. It was afterwards ascertained that the phenomenon observed in the case of mustard oil was due to the presence of the colouring matter present in the oil and was not due to any glyceride in the oil itself.

6. *Consistency and Viscosity*:—On a very careful investigation and examination of the data available on this

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- (1) Arbeiten a. d. Kaiserli, Gesundheitsamt, 1907, 26, 3, 444; 1908, 373;
 - (2) Zt. f. Unters. d. Nohr Genuss. 1913, 25, 367.
 - (3) Elsdon: *Edible Oils and Fats*, p. 400;
 - (4) Harris: *Analyst*, 1906, 31, 353;
 - (5) Wanters, *Bull. Soc. Chim. Belg.* 1905, (19), 6.

subject, it was concluded that neither of these could be used with advantage for this purpose. "Viscosity of butter varies so much that a very considerable admixture of margarine might escape detection."¹

7. *Electrical Conductivity*:—The estimation of the electrical conductivity of oils and fats has been suggested by Palmieri² to detect adulteration in olive oil with some amount of success. Want of sufficient data in this connection renders the proposal inapplicable for solving this problem.

8. *Calorimetric Examination*:—this method is not likely to find much application as the meagre information furnished thereby is out of all proportion to the labour involved in making the determination.³

9. *The Solubility Method*:—The differences as regards solubility of butterfat and its possible adulterants in organic solvents are not so pronounced as to enable the detection of adulteration. Therefore, methods based on this principle and recommended by Horsley⁴ and others are either wanting in accuracy or are of a limited application. Crismer's⁵ method based upon the determination of the critical temperature of dissolution has been chiefly used by Belgian chemists in the examination of butter. According to Ewers,⁶ this method fails to be reliable when the butter is adulterated with either vegetable or animal fats.⁷ Valenta's test based on the solubility of oils and fats in

(1) Pullonoke: Apoth. Zeit. 11. 97; White and Twinning, J.S.CiL., 1913, 32. 804.

(2) Rend. della, Acc. di Napoli. 1881.

(3) Lewkowitsch, J. Chem. Tech. and Anal. of oils, fats and waxes Vol. I, ed. 5 p. 373.

(4) Chem. News, 1861, 230.

(5) Bull. de a Assoc. Belge des Chimistes, 1895, 9, 71, 143; 1896, 9, 339; 10, 312; also, Liege Congress, 1905, section I, p. 323.

(6) Jour. of Soc. Chem. Ind. 1884, 643.

(7) Milchwisentralbl, 1910, I, 154.

acetic acid also fails in its purpose for want of any concordant and accurate results. It should be borne in mind in this connection that all these methods are affected by the presence of free fatty acids which alter the solubility and nullify the results. Serious discrepancies are likely to arise in case of Valenta's test, owing, firstly to the use of acetic acid of varying strengths and secondly, to the influence of the presence of free fatty acids in the oil under examination.¹

The more recent methods depending upon the ether solubility of the glycerides have been suggested by Amberger² and by Seidenberg.³ The method of the latter depends upon the solution of the fat in a mixture of two or more solvents, one of which is more volatile than the other and has a greater solvent action upon the glycerides. The great disadvantage of these processes is the large amount of solvent which is necessary and also the differences in results likely to be caused by small differences in manipulation. The trial given by the present authors to all such methods do not prove their superiority over the Valenta-test.

10. *Precipitation Methods* :—Mr. P. B. Sanyal⁴ has employed this method for the detection of the adulteration of butterfat. In the author's own words "The tests are based on the solubility of butterfat in two definite mixtures of dry acetic ether and 93 per cent. alcohol under strictly controlled conditions of concentration and

(1) Allen, J.S.C.I., 1886, 5, 282, Hoton, Thomson and Ballantyne, J.S.C.I., 1891, 10, 233, Chattaway. Pearmin and Moor, Analyst, 1894, 19, 155; Jen, Freyer, and Weston, Ibid, 43, 3,

(2) J.S.C.I., 1916, 35, 1077.

(3) Ibid. 1917. 36, 1138, 1918, 37, 633A ;

(4) Sanyal, P. Adulteration of butter and ghee, with animal fat and vegetable ghee, and its detection 1929, published by the Imperial Agricultural Research Institute, Pusa, India.

temperature and the insolubility of the glycerides of higher fatty acids of animal fats in it''¹. Test No. I is based on dissolving one gram of melted fat in three c. c. of dry acetic ether and adding four c. c. of 93% alcohol to it at 30°C. and leaving the mixture in a bath of the same temperature for half an hour². As stated by the author himself, this test fails :—

- (i) In the case of butter fat prepared from buffaloes fed on cotton seed cakes³.
- (ii) In the case of an adulteration of butter fat by animal fat below 5 per cent⁴.
- (iii) In the case of adulterations exceeding 25 per cent where irregularities are said to occur⁵.

Test No. 2 consists of reversing the proportions of the reagents used and allowing the other conditions as before⁶. Phytosteryl-acetate test has been recommended to distinguish between the animal or vegetable origin of the adulterant⁷.

If the adulterant is vegetable ghee which can be a hydrogenated oil or fat of animal or vegetable origin, the detection of this either by the phytosteryl-acetate or cholesteryl acetate fails under the following circumstances. The test stands in the case of samples of oils and fats which are hydrogenated according to the method of Wilbuschewitsch-Normann, at

(1) Sanyal, P. Adulteration of butter and ghee, with animal fat and vegetable ghee, and its detection, 1929, published by the Imperial Agricultural Research Institute, Pusa, India, p. 154;

(2) Ibid.

(3) Ibid. 152;

(4) Ibid p. 154;

(5) Ibid.

(6) Sanyal, P. Adulteration of butter and ghee, with animal fat and vegetable ghee, and its detection, p. 155;

(7) Ibid p. 153;

comparatively low temperatures. By hydrogenating at 200°C, 75 per cent. of the cholesterol is converted into a resinous mass, hydrogenation at 200°C. leaves the phytosterin still intact, while at 250°C., it is so completely changed that it gives rise to a hydrocarbon melting at 102-103°C.¹ Any inferences, therefore, with regard to the presence of the animal or vegetable nature of the oil or fat when vegetable ghee is present must be very carefully drawn.

The author has pointed out himself that the adulteration of butter fat below 15 per cent, of animal fat is undetectable by test No. 2 given above. Further, it is also stated that the quantities of precipitates obtained by tests 1 and 2, with the same sample, vary considerably so that unless the complete history of the sample of the ghee is known, it is not possible to adopt these tests for the quantitative determination of the extent of the adulteration.² This is surely demanding too much. With test 2, no precipitate appears in the case of adulteration, the author states, with vegetable ghee unless the butter fat is adulterated to the extent of more than 50 per cent.³ The summary of inferences from this paper (which particularly deals with question of adulteration) is as follows :—

- (i) The test fails to give any quantitative results in case the previous history of the ghee under examination is not communicated to the analyst ;
- (ii) The test fails if the animal is fed on cotton seed cake which happens to be quite a nourishing food for cattle and is largely used ;

(1) Holde- Bleyberg : Kohlenwassertoffole und Fette 6th Ed p. 635 ;

(2) Ibid p. 153.

(3) Sanyal, p. Adulteration of Butter and Ghee, with animal fat and vegetable ghee, and its detection, p. 153.

(iii) The test fails in the case of vegetable ghee if the adulteration happens to be below 50 percent *i.e.* up to about 50% anybody could adulterate without being detected ;

(iv) Test No. 1 does not indicate the presence of adulteration with animal fat below 5 per cent.

From the above it is evident that the tests mentioned in this paper can hardly be recommended for universal application.

11. *Iodine Value*.—As is clear from the characteristics of the butterfat, the difference in the maximum and minimum iodine values of butterfat covers a very wide range. These great variations in the numbers prohibit its use in the examination of the adulteration of butterfat when taken alone.¹ This can be employed, however, as a handmaid to other methods, in special cases of doubt. The amount of unsaturated fatty acids, in butterfat, it should be remembered, is greatly influenced by the kind of the oil or fat given to the animal with the food.² Exceedingly low iodine values are obtained in the case of butterfats from cows fed on turnips and turnip leaves.³ From the above remarks, it is clear that the extent of the iodine value (which is due to the unsaturated fatty acids and their glycerides) does not enable us to draw any definite or final inference.

12. *Hehner Value*.—The determination of the Hehner value and the mean molecular weight of the insoluble fatty acids are not of much help. Even if the normal numbers are found, they afford no proof of the purity

(1) Lewkowitsch, J. Chem. Tech. and Ana. of oils, fats and waxes, Ed. 5 vol. 2, p. 853 and p. 804.

(2) Lewkowitech, J. Chem. Tech. and Ana. of oils, fats and waxes Ed. 5, vol. 2 p. 855.

(3) Lewkowitsch, J. Chem. Tech. and Ana. of oils, Fats, and Waxes, ed. 5, Vol. 2, p. 855.

or otherwise of the sample. An unavoidable source of error lies in washing out a larger or smaller amount of Lauric acid from the insoluble fatty acids.¹

13. *Colour Reactions*—There are certain colour tests applicable to certain individual oils, as for instance, Halphen's test for cotton seed oil, Baudouin's and Soltsien's tests for sesame oil etc. The colour reactions can be used to detect adulterants which have not been detected by other methods. The drawback of the colour reactions is that they may only be applied to indicate the presence or absence of certain single fats or groups of fats. Therefore, a negative result of one special reaction does not indicate the purity of the butter fat. Such tests, however, can be employed to confirm the information obtained by other means.

14. *Cryoscopic Method*.—Pailheret² recommends Raoult's Cryoscopic method for determining the purity of butter-fat. The method deserves a careful trial and may reveal some tangible solution if modified to the special case of butterfat.

15. *Ewer*³ and *Fendler*⁴ have proposed a method of detecting adulteration in butterfat by determining the solubility of the Barium and the Magnesium salts of the fatty acids. It seems, however, to be far from satisfactory as pointed out by Polenske.⁵

16. *X-Ray Examination*.—An Allahabad daily paper once reported that Mr. Saligram Bhargava of Allahabad University had observed "a beautiful halo, surrounding the

(1) Lewkowitsch J. Chem. Tech. and Ana. of oils, fats and waxes, ed. 5, vol. 2., p. 855 ; Ludwig and Haupt. Zeits f. Unters. d. Nahr u. Genussom., 1907, XIII, 605 ; Heri. Ibid, 1608, XV, 529 ;

(2) Bull. Soc. Chim. 1909, 425 ;

(3) Milchw. Zentralbl. 1910, I, 154 ;

(4) Zeit. f. Oeffentl. Chem 1910, 16, 152 ;

(5) Arb. a. d. Kais. Gesundheitsamt, 1911. 38, 408.

central spot obtained on a photographic plate exposed to the Aluminium window of an X-Ray tube on which a drop of ghee is placed." As the observation was only partially reported, the author was requested to give more particulars. Consequently, after referring to the phenomenon mentioned above, the author says, "We have not been able to subject adulterants to the same process so as to find out whether the ring due to ghee was its characteristic ring. Unless this is done, it is impossible to say that the test is a successful one." Any further comment on this explanation is unnecessary.

17. *Kirschner Value*¹ :—Jensen² suggested that an estimation of the amount of caprylic acid in the Reichert Meissl distillate would indicate the presence of coconut oil in butter, since caprylic acid occurs to a far greater extent in coconut oil than in butterfat. On this basis, Kirschner evolved out a process to estimate the content of coconut oil. In this respect, it has no doubt proved very helpful. An attempt was made by Bolton, Richmond, Revis³ and Cranfield⁴ to define the relationship existing between the Kirschner and the Polenske values of butterfat but no very definite and reliable relationship has been established. (For details, see Chapter VII).

The Barium method of Ave-Lallement⁵ has been specially useful in indicating the adulteration of butter with lard, oleo and vegetable oils but not with the oils of the coconut series. Again, according to the author himself, it breaks down in the case of over heated or rancid butter.

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1. Zeit. f. Nahr. u. Genu. 9, 65, 1905.
 2. Farmaceutisk Titende, 1903, p. 385.
 3. Analyst, 37, 183, 1919.
 4. Ibid. 44, 166, 1919 :
 5. Zeit, f. Nahr. u. Genu., 14, 317, 1907 :

A positive Ave-Lallement's value cannot be a criterion of adulteration. Even in Ave-Lallement's original results¹ in which all the values were negative, the lowest R. M. V. was 24.6 and the curve passed over, if continued, to positive values at a R. M. V. of 25. Brownlee² has thrown considerable doubt on the validity of the claim that Ave-Lallement's value may be used as a criterion of genuineness for butter with abnormally low R. M. Values. But it breaks down according to the author himself, in the case of overheated or rancid Butter. (See Chapter VII).

Similar methods have been suggested by Blichfeldt,³ Shrewsbury and Knapp,⁴ Burultt and Revis⁵ and Elsdon⁶ etc. All these along with the original one of Kirschner have not been able to keep pace with the ever-increasing knowledge and skill of the sophistical or.

18 *Rapid Tests* :—There is a universal and pressing demand for a rapid test that could be applied anywhere in the market place by even a layman so that adulteration could be rendered easily detectable without evolving any very elaborate measures. It would be certainly commendable if any such method could be found out. Some such tests have been proposed from time to time by Hinks, Tocher, Halphen,⁷ Boudouin,⁸ Boulle,⁹ Bellier, R. B. Seth,¹⁰ Harisingh,¹¹ and others. The composition of butterfat is

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1. Z. Nahr. Genussm., 1907 ; 4,321.
 2. Proc. Royal Dublin Soc. 18. (N.S.) 49.
 3. J.S.C.I., 29, 792. 1910 ;
 4. Analyst, 35, 385, 1910 ;
 5. Ibid. 38. 255, 1913 ;
 6. Ibid. 42, 298, 1917 ;
 7. Lewkowitsch, J. Chem. Tech. and Anal. of oils, fats and waxes, Ed. 1, Vol. 2, p. 203 ;
 8. Ibid p. 220 ;
 9. Comts Rend. 11-112, 105-106 ;
 10. & 11. By private communications ;

too complicated to be handled and solved so easily. The methods proposed by these authors have, of course, not been found on actual experimentation to be of sufficient value.

Dr. Dunn and Dr. Pandya¹ of the U. P. Public Hygiene Institute, Lucknow, have recommended a method wherein the sample of Ghee under examination is subjected to the Welman's Test. The trial given to the same in this Laboratory has led the present authors to the following conclusion :—

(i) Green colour was formed in the case of butterfats obtained from cows fed on cotton seed cakes.

(ii) Castor oil gave reddish brown colour.

(iii) With Talgol and Candelite, (two hydrogenated fats) a dark grey colour was invariably observed.

The test, therefore, is both unreliable and misleading. That is also the experience of Seiger, Lewkowitsch, Kuhn and Halfpaap.²

19. *Aniline Point Determination*³ :—The claim put for this is also not borne out by any convincing experimental figures.

20. *Diffusion Method*⁴ :—Separation of butterfat by diffusion method has not been found to give any encouraging results like so many other common methods.

21. *Transition Points of Mixtures of Cow's Butter and Cacao Butter* :—D. W. Horn and M. A. Wilson⁵ have found out that relation between the transition point and % of

1. Dunn and Pandya : The Chemistry and Bacteriology of Public Health, p. 81.

2. Analyst, 1906, 31, 413;

3. C. J. Katrakis and J. G. Megaloikonomos, Praktika, 1930, 5, 267-269; Chem. Zentr. 1932, 1, 1459.

4. Hacket and Crowley : J.S.C.I., 1925, 44, B. 112;

5. Amer. J. Pharm., 1934, 106, 59-61;

cow's butter (if less than 70 %) is linear. Its application to the problem of detection of adulteration in butterfat is yet to be shown.

22. *Reichert Meissl and Polenske Values* :—The determination of these values is a distinct advancement over the previous methods although it varies within very wide limits. The minimum and maximum values of Reichert Meissl for butters even from single cows have been found to vary between 16·8 and 40·0 respectively.¹ In a particular sample of pure butterfat, a Reichert Meissl value so low as 13 has been obtained. Still the Reichert Meissl value furnishes a quick and sufficiently reliable indication of the presence of adulterants in butterfat'. Depending upon the nature³ of the food, season of the milking, weather condition,⁴ the period of lactation⁵ and the idiosyncrasy of the cow⁶ etc., the Reichert Meissl and the Reichert Polenske values vary within sufficiently wide limits⁷. Mention may be made here of some intelligent attempts made to increase the Reichert Meissl value by adulterating butterfat with volatile and water-soluble substances like acetin and amyl-acetate. The longer the period given for saponification in this process, the higher is the Reichert Meissl value obtained due to the depolymerisation of the acids. Saponification with glycerol-potash

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1. 46th Report of Danish Agricultural Laboratory :
 2. Tinkler and Masters : Applied Chemistry. p. 60 ;
 3. Spallanzani and Pizzi : Staz. Sper. Agrar. Ital., 38, 257 ; Baumert and Falke, cp. also A. Ruffin. Ann., de Chim. Anal., 18 9, iv. 383 ;
 4. Siegfeld, Zeits. f. Unters. d. Nahrgrs. u. Genussm., 1909, xvii, 179 ;
 5. Vieth, Milchzeit., 1899, 785 ; Spallanzani and Pizzi, Ibid, 1889 461, 483 ; Paal and Amberg, r, Zeits. f. Unters. d. Nahrgrs. u. Genussm., 1909, xvii, 41 ; Smetham, Analyst, 1909, 804 ; Swaving, Landw. Versuchsstat., 1891, 127 ; Swaving, Zeits. f. Unters. d. Nahrgrs. u. Genussm 1905, x. 81, 1906, xi, 595 ;
 6. Enecke, Mitt. d. Landw. Inst., d. Univ. Breslau, 1903, 2, 559 ; Fischer, Zeits., f. Unters., d. Nahrgrs. u. Genussm., 1905, x, 338 ;
 7. Lewkowitsch, J. Chem. Tech. and Ana of oils, fats and waxes Ed 5, Vol. 2, p. 822, T. K. Ghosh, Analyst, 1920, 444 ;

yields a lower value than alcoholic saponification, probably because at high temperatures some of the acids are decomposed¹.

Some workers suggest that Reichert-Meissl value of 6.28 may be taken as a *test of purity* because it represents the *average*. But obviously *purity* and *average* are not equivalent in such cases and, therefore, the above cannot be laid down as a standard^{2, 3}.

Polenske tried to establish a certain relation between the Reichert-Meissl and the Reichert-Polenske values, the idea being that any variation from this ratio was to be taken as an indication to prove adulteration. From actual experimental data, however, this ratio-inference is found not to hold good. Several other workers also have expressed a similar view. This estimation, therefore, is of much help only in confirming doubtful cases. In the words of Elsdon⁴ "Like many other processes, Reichert-Polenske value is an excellent slave *but a bad master* and this fact should never be lost sight of when its indications come up for judgement."

An attempt has also been made to determine the butter content from the Reichert-Meissl and Saponification values⁵; but its application is limited, of course usefully, only for the detection and determination of butter in margarines. The accuracy of this calculation has been confirmed by Pritzker⁶. Further attempt has been made to extend this observation to a universal examination of butter⁷.

1. J. Delaite and J. Legrand, Bull. Soc. Chim. belg, 1906, 20, 230-35.
2. Soyde and Woy. Chem. Ztg., 18, 1894, 906;
3. Elsdon: Edible Oils and Fats, p. 385;
4. Ibid., p. 384;
5. Kuhlmann and J. Grossfeld: Z. Unters. Lebensmittel 1926 (10) 38;
6. Pritzker: Z. Unters. Leb. 1929 (58) 592;
7. Grossfeld: Z. Unters. Leb., 1930 (59) 104;

23. *New Distinguishing Value for Milk Fat* :— J. Kublmann and Grossfeld¹ have devised a method to extend the differentiation of volatile fatty acids of butterfat effected by Gilmour² by eliminating the influence of the caprylic acid. For this purpose the distillate obtained as in the Reichert-Meißl determination is salted out with Na_2SO_4 and sufficient K-Caprylate (Coconut soap solution) to form a saturated solution. Samples of butter of different origin gave Butyric acid values ranging from 18.6 to 22.0 whilst coconut oil gave value of 0.8 to 1.0 probably due to a volatile acid other than caprylic (Caproic?). The Butyric acid value stands in a definite relationship to the Reichert-Meißl value. If the former is divided by the latter, the average quotient is 0.745. For an accurate determination of Butter fat in an unknown fat, the Butyric acid value and Saponification value are determined and from these values the amount of coconut oil present can be calculated while the original amount of Butyric acid value corresponds to the Butter fat.

24. *Caprylic Number*³ : The determination of this number is now more thoroughly covered by the A and B values to be described later on.

25. *Aylene Number*⁴ : This method requires further confirmation and examination from the point of view of its actual application to the present problem.

26. *Fluorescence Analysis*⁵ : An account of this is given from the original publications of the experimenters

1. Z. Unters. Lebensm., 1926, 51, 21-42 ;

2. Analyst 1925, 50, 276 ;

3. Zeits. Unters. Lebensm. 1928, (55) 354 ;

4. Van Raalte : Z. Unters. Lebensm., 1927 (53) 236 ;

5. Prof. P. W. Danckwortt, "Lumineszenz-Analyse im filtrierten ultravioletten Licht."; C. Ellis & Wells, "The Chemical Action of Ultra-Violet Rays."; R. S. Morgan & K. McLennan, "The Fluorescence of Some Vitamin A Containing Fats."; W. D. McGillivray, "Ultraviolet Rays and Their Properties."

as follows : Morgan and McLennan have devised a method of measuring fluorescence by an adaption of Guild's colorimeter, and have applied this to tests of butters and margarines under the Hanovia Analytic Lamp. They confirm Peacock's reported association between vitamin A and a golden fluorescence in fats. Croners's work on oils (particularly soya, colza and sesamum) is most interesting. Exposed to the rays of the Analytic lamp, in an open vessel, the oils show in part a characteristic fluorescence on the upper surface, and in part a most peculiar coloration of the fluid itself. Typical changes occur in the fluorescence if the oils are heated beforehand. Admixture of vegetable and mineral oils also alters the fluorescence. Indistinct mixed colours indicate a combination of different vegetable or animal oils.

Admixture of margarine in butter of 25 per cent or over can be ascertained through the bluish fluorescence of the coconut fat contained in the margarine.

Thorough tests have been made on lard by Feder and Rath, and Van Raalte. Refined pig's fat is fluorescent but lard is not. A peculiar and characteristic fluorescence is given by white grease and fats containing it ; according to Bengen, the presence of even less than 1/1000 per cent can be ascertained with certainty.

In view of all the above mentioned observations by various workers, it is really worth while to give a very fair trial to this type of analysis for detecting adulterants in butterfat.

27. *Lwkwitsch* has suggested the idea of making a quantitative determination of insoluble fatty acids like stearic, arachidic and myristic either by distilling them in vacuum or preferably by distilling their methyl esters.

The content of stearic acid in butter fat amounts to only 0.5 per cent while it is more than 25 per cent in other fats.¹ This determination of the exact content of stearic and other acids is by no means an easy process. Not only does it demand high technique and skill on the part of the worker but the separation of pure acids from their mixtures is rather difficult. In the case of the determination of the presence of cerotic acid in ground nut oil, for example, as many as two kilos of oil were required to get about 2 gms. of pure cerotic acid by the method suggested by Lewkowitsch.² This method, in view of the present knowledge of oil-analytical chemistry, is neither easy nor practical.

28. *Refractive Dispersion Method*:—The dispersions of oils and butterfat were first investigated by Szala'nyi³ and later on by Freyer and Weston⁴. But it was left to Messrs V. T. Athavale and S. K. K. Jatkar⁵ to study this method with special reference to its application in the detection of adulteration in butterfat. Their conclusions are as follows :

“1. The refractive dispersions of butterfats from different sources have been measured on a Pulfrich Refractometer and the dispersion constants are found to be sufficiently different from those of vegetable oils to account for the characteristic colour-fringes on a simple type of Butyro-refractometer.

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1. Lewkowitsch, J. Chem. Tech. and Ana. of Oils, Fats and Waxes, vol. 2, p. 858.
 2. Holde and Godbole : Zur Kenntnis Der Hochstmolekularen Gesättigten Sauren des *Arachis Oles*.
 3. Biochem. Zts., 1914, 66, 173-176.
 4. Analyst, 1918, 311-317.
 5. Journal of the Indian Institute of Science, Vol. 1A, part 3, pp. 1-25.

“It should be possible to detect adulteration of ghee by measurement of both refractive index and dispersion on a Pulfrich refractometer using the green and violet lines of the Mercury arc.

“2. Mixtures of oils can be prepared to give the same refractive index as that of ghee, but it will be difficult to get the same dispersion, especially in view of the fact that oils of lower refractive index and consequently lower dispersion are usually employed for adulteration.”

These observations of dispersion constants present further important support to the colour-fringes as observed by the present authors. Further experiments on the dispersion constants are progressing and there is no doubt that this method, if properly investigated, should throw great light on the problem of detection of adulteration in butterfat.

29. *Differentiation of oils by Enzymic Hydrolysis*:—The copper-soap test described by Carnot and Mauban¹ for the detection of lipases has been modified and extended to the differentiation of oils and fats by Messrs K. V. Giri and P. N. Bhargava.² The method takes advantage of the difference in the fatty acids of oils hydrolysed by lipase which form soaps with copper sulphate according to the Agar Plate method. The authors of the above-mentioned paper have summarised their results as follows:—

“It was found that the colour of the adulterated sample, on comparison with the colour of the central zone produced by pure ghee, was changed from dirty yellowish to bluish green, the intensity depending on the concentration of the adulterant. The addition of any of the oils (like coconut, sesame, groundnut, mahuwa, safflower, lard and

(1) Compt. rend. soc. biol. 81, 98, 1918.

(2) Ind. and Eng. Chem., Analytical Edition, Vol. 9, page 395, Aug. 15, 1937.

castor) always had the effect of increasing the bluish green tint, thereby altering the colour of the central zone characteristic of pure ghee. The admixture of 20% and more of sesame, groundnut, coconut, lard and other oils with a sample of pure butterfat could readily be detected by this method.

“The depth of bluish green colour of the central zone is to a certain extent proportional to the amount of the other oil present in butterfat, and by making comparative tests with mixtures of butterfat and the oils in various proportions some idea as to the amount of the adulterant can be obtained.

“Samples of pure ghee stored in bottles and tested after about a year produced zones having the same colour as that of a pure fresh sample. The entire surface of the agar plate was coloured light green when flooded with copper sulphate, however, probably because of the presence of fatty acids formed in the sample after storage. It is hoped that with the help of a tintometer the test can be standardised in terms of the colour units and that a standard colour unit for pure butterfat can be established.”

It is recommended that further exhaustive experiments should be undertaken on a large number of not only pure butterfats but also adulterated samples of various types and proportions before the method is standardised. Further data on the use of this method by the authors are welcome.

CHAPTER VII

Evolution in the Chemical Methods of Ghee Analysis from Reichert Meissl Value (1879) to Butyric Acid Number (1927), and its Modification (1935).

(Reprinted from Science and Culture April, 1937.)

Ghee or butterfat is the fatty portion separated from the milk of a cow or a buffalo. It is well known that ghee forms an important and necessary nourishing food article, especially to the vegetarian in India, and very large quantities of this are consumed. Due to the high price of butterfat as compared with the other oils and fats, fraudulent adulteration of butterfat is profitably carried on by dealers, on a very large scale. The problem of determining the purity of butterfat is thus of very great importance. The adulterants¹ mostly used are cocoanut oil, mohua, hydrogenated oils, etc. Like all other oils and fats, butterfat is a mixture of glycerides of various fatty acids. The proportion of the constituent fatty acids in the total fatty acids of an average sample of butterfat is given below.

Table No. 17.

Name of acid.	Proportion*	Butterfat**	
	Average.	Cow.	Buffalo.
Butyric	.. 3.1 — 3.4	4.0	4.0
Caproic	.. 1.7 — 1.9	2.0	2.0

* Hilditch & coworkers: *Analyst* 54, 75, 1929; 55, 75, 1930.

** Sadgopal: (Extract from the *Thesis*, submitted to the B. H. University, for the Doctorate degree.)

1. Godbole and Sadgopal: *Butterfat*, p. 9, 1930.

Table No. 17.—(concluded.)

Caprylic	..	0·8 — 0·9	0·9	0·9
Capric	...	1·9 — 2·3	2·0	2·0
Lauric	..	3·1 — 4·3	4·5	4·0
Myristic	..	9·7 — 10·8	10·0	9·0
Palmitic	..	27·6 — 28·4	26·4	31·0
Stearic	..	8·5 — 12·2	10·0	12·0
Oleic	..	33·1 — 36·4	34·5	30·0
Linoleic	..	3·7 — 5·4	5·0	4·0
Arachidic	..	0·5 — 1·0		
Unsaponifiable	..		1·0	0·45
Total	99·9	99·35

From the data given above, it can be seen that butterfat differs from most of the other oils and fats in two respects. Firstly the content of the lower fatty acids up to C_{10} is high, and secondly butyric acid is present to the extent of about 3·5 %. Most of the chemical methods for the estimation of butterfat are based on these two important and characteristic values, besides the physical method of the determination of the refractive index also.¹

One of the important methods for the separate estimation of the individual constituent fatty acids of an oil or fat, is the method of separation of the methyl esters of the fatty acids by fractional distillation under reduced pressure. This method is, however, very laborious and requires a large quantity of the sample under examination. The first practical method proposed for such work (estimation of butter-fat, cocoanut oil, palmkernel oil etc.), which can be carried out on a small sample, was enunciated and carried out by Reichert.² This method with the modifications of Meissl and Polenske is widely used for the estimation of butterfat, cocoanut oil and palmkernel oil. The method is based on the estimation of a definite

1. Godbole and Sadgopal *Butterfat* p. 27, 1930.

2. Reichert : *Ztschr. analyst. Chem.* 18, 86, 1875.

portion of the steam-volatile fatty acids, obtained under definite empirical conditions. The detailed method is given below.

Estimation of the Reichert Meissl and Polenske Values¹

Definition : The Reichert Meissl value is represented by the number of C.C. of N/10 alkali required to neutralize the steam-volatile and water-soluble fatty acids obtained by the prescribed method, from exactly 5 gms. of the fat

The Polenske value is represented by the number of C.C. of N/10 alkali required to neutralize the steam-volatile and water-insoluble fatty acids obtained by the same procedure from exactly 5 gms. of the fat or oil.

Procedure : Exactly 5 gms. of the sample are weighed out into a 300 C.C. round bottomed Jena flask, and are saponified over a direct flame, with 2-4 C.C. glycerine and 2 C.C. of aqueous KOH (50%) solution. The saponification is carried out with care and *without overheating*, until the contents of the flask are quite clear. When the contents have cooled to 80°C., 90 C.C. of freshly boiled water at the same temperature are added. To this, 50 C.C. of dilute sulphuric acid (25 C.C. of con. H₂S O₄ per litre) and 0.6 to 0.7 gm. of pumice powder are added. The flask is immediately closed and the contents are distilled in the prescribed apparatus in such a way that 110 C.C. of the distillate are obtained in 19 to 21 minutes. When exactly 110 C.C. are received, the flame is removed and the receiver is replaced by another clean vessel. The receiver is dipped in water at 15°C. for 10 minutes. The distillate is then shaken thoroughly and filtered through a smooth dry filter-paper having a diameter of 8 cms. If the filtrate is not perfectly clear, it is shaken with a little kieselguhr and

1. Wizoff. *Einheitliche Untersuchungsmethoden für die Fett- und Wachs Industrie*, 1930, p. 85-87.

refiltered through the same filter paper. 100 C.C. of the filtrate are titrated with N/10 alkali using 3 to 4 drops of a neutral alcoholic 1% solution of phenolphthalein as an indicator. A blank experiment is also carried out.

Calculation : R. M. value = $1.1(a-b)$ where a = C.C. of N/10 alkali used in the main experiment and b = c.c. of N/10 alkali used in the blank experiment.

Procedure for R. P. Value

In order to remove completely the water-soluble fatty acids, the condenser, the second receiver and the first receiver are washed successively three times, using 15 c. c. of water each time. Each 15 C.C. of wash water are filtered through the filterpaper previously used. In order to dissolve out the remaining water-insoluble fatty acids, the above mentioned vessels are washed with 90% neutral alcohol in an exactly similar manner, and the wash-alcohol passed each time through the filter paper. It is to be remembered that the second and the third 15 C.C. of alcohol are transferred to the filter paper, only when the first and second have completely drained out of the filter paper. The total alcoholic solution is titrated with N/10 alkali as before.

Calculation : Polenske Value = C.C. of N/10 alkali req. for the titration.

The drawbacks and shortcomings of the R.M. and R. P. values as applied to Butterfat analysis.¹

“The classical method for estimating the purity of butterfat and cocoanut oil is the determination of the R. M. and R. P. values. The procedure of the method is such that actual results obtained are quite different from those theoretically aimed at.

1. J. Grossfeld : *Fette und Seifen*, July, 1936, pp. 100-101.

The distillation of the fatty acids proceeds from beginning to end in a three phase system : Aqueous solution/Insoluble fattyacids/vapour phase.

The insoluble fatty acids behave as a powerful solvent towards the fatty acids in the aqueous solution. The result is that the volatilization of the water-soluble fatty acids is checked. In a similar manner the volatilization of the water-insoluble fatty acids is also hindered. The yield of the total volatile fatty acids is thus much less than the theoretical amount expected. The agitation of the water-insoluble fatty acids by the stream of water vapour, which takes place while the distillation proceeds, has an undesirable effect. Lauric and myristic acids distil over to an appreciable extent, while palmitic acid can also be detected in the distillate. Cocoa butter which does not contain even traces of lower fatty acids, gives a small Polenske value due to the reasons mentioned above.

“The separation of the watersoluble fatty acids from the water insoluble ones is done by simple filtration, with the result that the insoluble portion contains traces of butyric and caproic acids, while the soluble portion contains most of the butyric and caproic acids, appreciable quantities of caprylic acid and measurable quantities of capric acid. Thus butyric, caproic, caprylic, and capric acids are estimated partly in the soluble portion and partly in the insoluble portion. The size and shape of the distilling arrangement greatly influences the R. M. and R. P. values, which is a great disadvantage.

In order to effect a more complete separation of the fatty acids which are characteristic for butterfat and cocoanut oil, two artifices are generally employed.

(1) Separation of the water-soluble fatty acids from the water-insoluble ones by filtration before distillation.

(2) Separation of the higher fatty acids by precipitation of their Mg soaps by means of $MgSO_4$ solution.

The first artifice is employed with a special modification by Grossfeld for his *Butyric acid value*.¹

The second artifice has been employed by S. H. Bertram and his co-workers for estimating the *A and B values*.²

From Reichert Meissl to Butyric acid value.

After Reichert, a good many workers in the field have devised methods for the estimation of butterfat and cocoanut oil, depending upon more or less similar principles. Most of the earlier methods have been given up in favour of the more recent improved methods. All these methods³ are given below in the order of their sequence.

Methods based on the separation of the silver soaps.

1. Caprylic acid value	Jensen	1905
2. Capric acid value		
3. <i>Kirschner value</i>	Kirschner	1905
4. First and second Silver value	Wijsmann	1906
5. First and second caprylic value.	Dons	1907

Other metallic salts.

6. Copper value	Bellier	1907
7. Cadmium value	Paal & Amberger	1909
8. Baryta value	Firtsch	1907
	Ave Lallement	
	Ewers	1910
6. <i>A and B values</i>	Bertram, Bos and Verhagen	1923
19. <i>Butyric acid value</i>	J. Grossfeld	1927

1. J. Grossfeld and J. Kuhlmann: *Z. Untersed Lebensmittel*, 51, 31, 1926.

2. S. H. Bertram, H. G. Bos, and F. Verhagen: *Chem. Weekbl.*, 20, 60, 1923; *Chem. Zentralbl.* 50, 244, 1925.

3. Grun: *Analyse der Fette und Wachse*, vol. 1. p. 169, 1925.

The R. M. and R. P. values and the Kirschner¹ value have been upto now the classical methods for the estimation of butter fat. The description of the Kirschner value also is therefore given below in detail.

The Kirschner value represents the number of C.C. of N/10 alkali required to neutralize the R. M. fatty acids (containing a specified quantity and obtained under definite conditions) the silver salts of which are soluble in aqueous solution. This value, therefore, primarily represents the amount of butyric acid contained in an oil or fat, but is liable to an error due to the presence of caproic acid. Caprylic acid and the high homologues are, however, without any influence.

Procedure : 100 c. c. of the distillate as obtained in the apparatus by the R. M. procedure are neutralized with N/10 baryta solution and 0.5 gm. of finely powdered silver sulphate is added. The mixture is allowed to remain for one hour with repeated shaking. The liquid is then filtered and 100 C.C. of the filtrate are transferred once again to the R. M. distilling apparatus. 35 C.C. of water are added along with 10 C.C. of dilute sulphuric acid (20.25 C.C. conc. H_2SO_4 in a litre) and a long piece of aluminium wire. 110 C.C. are then distilled in 20 minutes. 100 c. c. of the distillate are then titrated with N/10 NaOH solution, using phenol-phthalein as the indicator. A blank test is carried out in a similar way. The Kirschner value is calculated for 5 gms. of the fat as follows :—

a = C.C. of N/10 baryta solution required.

b = C.C. of N/10 NaOH solution required for the end titration minus that required for the blank titration.

1. Kirschner : *Z. Nahrungsm.* Bd. 9. p. 65, 1905; Grun : *Analyse der Fette und Wachse* vol. 1. p. 170 1925,

$$\text{The Kirschner value} = \frac{b \times 1.21 (100 + a)}{10}$$

The Kirschner value has often been described¹ as a value as useful as the B value. It is, however, not so since the determination of the Kirschner value is based on the estimation of the butyric acid obtained in the distillation by the R.M. method, the defects and inaccuracies of which have been discussed above. The A and B values are a development on all the older values including the Kirschner value. It has been pointed out in the discussion on the R. M. procedure that the separation of the lower fatty acids as water-soluble and water-insoluble ones involves certain fundamental errors. These errors are eliminated in the case of the fractional precipitation method as followed in the A and B value procedure.

One outstanding advantage of the A and B values over the similar and older values is the very narrow range found for pure samples of butterfat. A comparative table of the R. M., R. P. and A and B values of a number of butterfat samples of known purity, is given below.

Table No. 18.

A Tabular Statement for R. M., R. P. and A and B Values of the Same Samples of Butterfat.

Source.	R.M. value.	R.P. value.	A value.	B value.
Cow	.. 21.0	0.7	6.3	33.0
„	... 23.3	0.75	6.5	33.3
Buffalo	.. 24.6	0.85	6.8	35.0
Cow	.. 26.4	1.74	6.3	32.85
„	.. 27.0	1.8	6.4	33.5
„	.. 27.75	1.15	6.3	31.0
„	.. 28.6	0.85	6.2	33.0

1. Herbert Hawley, Current Science, May, 1936 pp. 815-817,

Table No. 18.—(concluded.)

„	..	29·2	1·1	6·2	33·3
Buffalo	..	29·85	1·05	6·4	33·0
Cow	..	30·76	0·9	6·3	33·2
„	..	31·0	1·5	6·2	34·0
Buffalo	..	32·5	1·75	6·5	35·5
„	..	33·55	1·35	6·35	35·0
„	..	34·0	1·35	6·5	34·0
„	...	35·0	1·6	6·6	35·5

Some critics of the A and B values have referred to results quoted by Koenig,¹ in order to show that the B values of certain samples of butterfat vary within wide limits.

Dr. Bleyberg referring to this quotation from Koenig writes to us as follows.

“ I have looked up Koenig’s book, at the library of the Deutsche Chemische Gessellschaft.

As to passages quoted by your critic, the following data occur on p. 720 of vol. 2 in the following form :

The A value for butterfat is 6·7—7·1.

The B value for butterfat is 26·7—43·1.

If you compare these figures with those given in Holde’s 6th edition, p. 623, table 151, you will see that they only represent a summary of Bertram’s own figures quoted in Holde’s table, but unfortunately Koenig’s summary contains two mistakes. 1) The lower limit (26·7) is obviously a misprint for 29·7; 2) Koenig entirely overlooks the fact that the extraordinarily high value 43·13 of butterfat No. 5 relates to *rancid* Australian butter and, therefore, under no circumstances ought to be

1. Koenig : Untersuchung landwirtschaftlich, u. land w. gewerblich wichtiger Stoff, Vol. II. p. 720.

included in the range of normal butter fats. *Consequently both of the limits given by Koenig for B values of butterfats are wrong.*"

The butyric acid value is a development on all the other values mentioned above. The procedure for the determination of this value is given below.

Butyric Acid Value.

The butyric acid value stands for the no. of C.C. of N/10 alkali required to neutralize the amount of acid obtained by distillation from 5 gms. of a fat which after saponification in aqueous solution is saturated with sodium sulphate and caprylic acid, and acidified with sulphuric acid.

Procedure : 5 gms of the fat under investigation are saponified with 2 C. C. of aqueous KOH solution (750 gms KOH in a litre), and 10 C. C. of glycerine in a 300 C. C. round bottomed flask, carefully over a free flame. The clear soap solution is allowed to cool a little and diluted with 100 C. C. of distilled water. After cooling the solution to about 20-30°C, 50 C. C. of dilute sulphuric acid (25 C. C. conc. H₂SO₄ in a litre) are added. Now 15 gms of anhydrous sodium sulphate are gradually added and dissolved in the mixture and 10 C. C. of a 10 % *cocoanut soap solution* are added.

Preparation of the cocoanut soap solution : 10 gms of pure cocoanut oil (known in Germany by the trade name of *Palmin*) are saponified with 4 C. C. of aqueous KOH solution of the same strength as that used above, and 10 C. C. of glycerine in a Jena flask. When the saponification is complete, the soap is allowed to cool and is then diluted to 100 C. C. with distilled water. (*We have used Tomco's pure cocoanut oil as a substitute*

for palmin and found it to be perfectly suited for the experiments.—AUTHORS.)

After adding the cocoanut soap solution, 0.1 gm of kieselguhr is added and the mixture is shaken for about 10 minutes. The mixture is then filtered through a dry fluted filter paper having very fine pores. The filtrate thus obtained must be perfectly clear.

125 C. C. of this filtrate and 50 C. C. of distilled water are taken in a 500 C. C. round bottomed distilling flask. Some pumice powder is added and the liquid is distilled until 110 C. C. of distillate are obtained. The distillate is titrated with N/10 alkali, (indicator being 1% phenolphthalein).

Butyric acid value = $1.4(a-b)$;

where a = C. C. required for the main titration,

and b = ,, ,, ,, ,, blank ,,

The butyric acid value for butterfat is about 20 and for cocoanut oil about 0.9. The butyric acid value corresponds to the proportion of butyric and caproic acids which are characteristic for butterfat. The separation of the butyric and caproic acids from the other acids is made sharp by practically saturating the solution with Na_2SO_4 , (K_2SO_4 also serves the purpose) and caprylic acid. The error which might have arisen from the caproic acid in the cocoanut soap is balanced by the same amount of caproic acid in the blank experiment.

A number of trials have recently been taken in the B. H. U. laboratory on the estimation of the *Butyric acid number*, with the help of some standard butters from the Indian market. The values obtained (in each case an average of two tests) are quoted below.

Table No. 19.

Some Experimental data on the Estimation of Butyric Acid Value.

Sample.	Butyric acid value.
1. From Polson's butter (August sample) 26.0
2. (September sample)	.. 25.7
3. (October sample) 25.7
4. (November sample)	.. 25.9
5. From Lord's butter	.. 25.1
6. ,, Dairy butter of the Agricultural Institute of Allahabad.	.. 24.1

It is interesting to note that whereas European samples of butter, as quoted by Grossfeld, give a butyric acid number of about 20, (and 18—20 as given on page 85 of the *Milchwirtschaftliches Taschenbuch—1936*), and (18.6 to 22, P. 61 of this edition) the Indian samples, examined so far, give an average of about 25.2. One possible explanation is to be found, perhaps, in the fact that most of the Indian cows and buffaloes are fed very largely on green grass and leaves (particularly in the months of August and September, and therefore they show a greater content of butyric and caproic acids; whereas, the European butters obtained from *cows* fed on *cakes*, yield more of the higher glycerides and less of the lower ones. As the number of samples tested so far in this laboratory is small, it would not be advisable to arrive at any final conclusions. Further work on this subject is in progress.

CHAPTER VIII

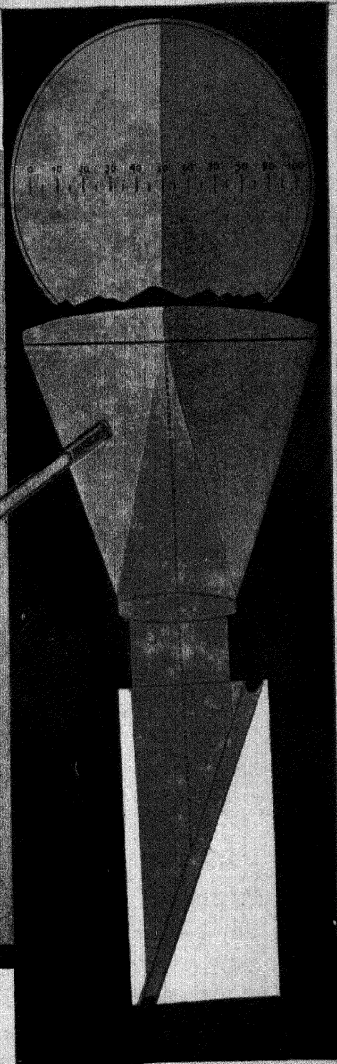
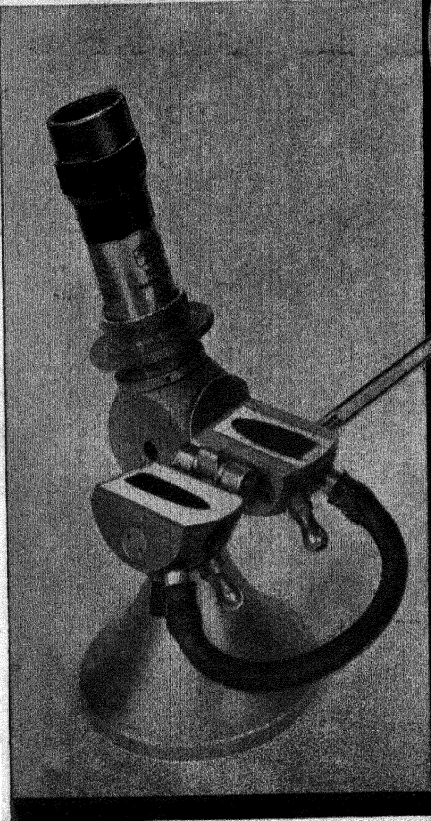
Butyro-refractometer and its application for the investigation of the adulteration in Butter fat.

Methods proposed for detecting and estimating the adulterants in Butterfat.

From the foregoing discussion, it is clear that no single method taken alone could help in determining the purity of butter-fat. For every value, as must have been observed, there is a range and it varies within comparatively large or narrow limits in different cases. It is found that perhaps the minimum range is found in the case of refractive index to which no reference is made in the previous parts intentionally. The use of the Butyro-refractometer, as recommended here, is not new in its application. As will be explained in this part, what is claimed in the use of the refractometer is the observation of *certain coloured fringes*. Butter fat as such has a very small range in the refractometric reading. Yet even in this small range, adulteration could be practised without its being always detected. The instrument is being used in India as a rapid and reliable method for the determination of the purity of butter-fat. It was further felt that not only could a certain amount of adulteration pass off undetected but it was possible to prepare a mixture of two oils and fats that could show the same refractive index as that of butter fat and yet contain no trace of it at all. That would be a serious position indeed. The unreliable

THE

ZEISS



BUTTER REFRACTOMETER

Sole Agents:—
ADAIR, DUTT & CO., LIMITED,
5, Dalhousie Square, Calcutta.

nature of the refractive index taken alone has been hinted long ago.¹

According to Elsdon^{2, 3} also, it is obvious that mixtures of coconut oil and animal fats can be prepared giving refraction figures covering the whole range of those for pure butterfat and therefore, as a sorting test, the refraction figure is now of no value.

Another factor which influences the refractive index of butter-fat is the feeding of the animal. Actual experiments have shown that by feeding animals with coconut cakes, a butter-fat giving a low refractometric value is obtained.⁴ Again by feeding with cotton-seed products, a rise in the refractive index has been observed.⁵

A very interesting observation has been made in the case of rancid samples of butter-fat. In such a case, it has been invariably found that abnormal refractometric figures are obtained with rancid butter-fats. According to the general rule, all such cases will have to be declared as adulterated ones. But really speaking, these abnormal figures are due entirely to chemical changes brought about by the process of rancidification. The following table will illustrate the behaviour of such samples which have been examined by the present authors.

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- (1) W. J. Indemans. Bull. Assoc. Belge. des Chim. 14, 10, 404-407,
 - (2) Edible Oils and Fats, p. 390 ;
 - (3) Chemist's Year Book, 1930, "Dairy Products";
 - (4) C. Pall and C. Amberger. Z. Untersuc. Nahr. Genuss, 1909, 17, 23—5
 - (5) C. M. Eckles and L. S. Palmer, Nissauri. Agric. Exp. Station Research Bull. No. 27, Dec, 1916. pp. 1—47. Bull. Agri. Intell. 1917, 8, 1021—22; Lindsay, Massachusetts State. Rep. 1900, 14.

Table No. 20.

Sample.	Acid value.	Butyro-Refractometric No. at 40°C	Acetyl value.	Godbole-Sadgopal Line.
1	0	42.0	0	Colourless.
2	4.3	42.4	5.1	Light Violet.
3	5.2	42.8	5.5	"
4	7.2	43.0	5.8	"
5	8.9	43.15	7.8	"
6	9.3	43.2	8.2	"
7	9.8	43.3	8.5	Violet
8	10.65	43.5	8.8	"
9	13.2	43.8	9.4	"
10	15.1	44.5	9.6	"
11	15.3	44.6	9.8	"
12	15.8	44.6	10.0	"
13	20.0	44.8	11.0	"
14	23.0	45.8	11.8	"

The inference directly drawn in view of the above-mentioned facts is that with the increase in the rancidity and the acetyl values, butyro-refractometric reading goes on increasing proportionately. This means that the glyceride molecule is giving up a part of its acid radical which is replaced by the OH (Hydroxyl) of the glycerine molecule. The molecules of the glycerides with the hydroxyl groups are already known to possess greater refractive indices than the pure glycerides. Thus it is possible to explain that rancid samples of butterfat, like adulterated samples, may show an abnormal refractometric values but that does not necessarily mean that the sample under examination is *adulterated* with foreign fat.

To confirm it still further, a nine year old sample of pure ghee got from a local Doctor in Ayurveda (Hindu Science of Medicine) was found to possess the following values :

Acid Value :	46.3
Butyro-refractometric number at 40°C	51.45
Acetyl value	29.3.

It is due to such complicated aspects that according to Lewkowitsch¹ and other researchers, "Determination of Refractive Index has now become of secondary importance". Again even the refractive index of insoluble fatty acids, which have narrower limits than the fats, are not reliable².

Therefore, attempts were directed at finding out a reliably qualitative method for detecting the adulteration in butter-fat. On a critical examination of the readings taken with the instrument, Butyro-Refractometer, certain characteristic colour fringes were observed when a series of oils and fats was subjected to the above test. Either a prominent blue fringe or a prominent orange red fringe was observable depending upon the constitution and composition of the oil or fat under investigation. As is well known, the oils and fats are classified into two broad groups, one class belonging to the cocount series having a saponification value ranging from 220 to 260 and the other class of oils and fats (excepting Japan Wax), having the saponification value ranging from 190 to 195. This difference is obviously due to the nature of the glycerides present in these two classes. In the first class where the saponification value is large, there is to be found a predominating portion of the glycerides of lower fatty acids ranging from C_4 to C_{12} . In the other class, there is the predominating element of the glycerides of fatty acids from C_{12} to C_{20} (both saturated and unsaturated). With this obvious difference in composition, the presence of the colour fringes is

(1) Lewkowitsch, *J. Chem. Tec. and Anal. of Oils, Fats, and Waxes*, 1922, 2, 833;

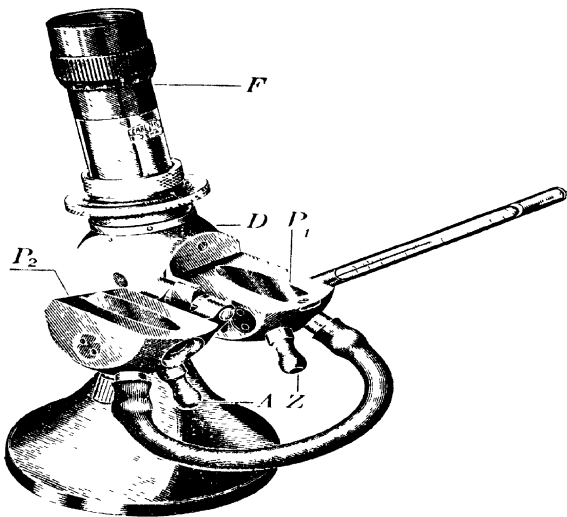
(2) *Ibid*, 834; Partheil & Velsen, *Arch. & Pharm*, 1900, 261; Raalte and Lichtenbelt, *Zeits., F. Unters. d. Nahrgu. U. Genussm*, 1912, iii, 82, cp. also Ludwig and Haupt, *Ibid.*, 1907. xii, 521; Dons, *Ibid*, 1907, xiii, 257, 1908, xv, 81; Ludwig. *Ibid.*, 1907. xiv, 208.

attributable to the phenomenon known as dispersion. The work was continued by taking known samples of pure ghee and adulterating it with known oils and it was found that according to the previous classification of oils and fats into two main classes, always the anticipated fringe of dispersion was observed. This is not an empirical result but is based on the constitutional character of the oils and fats. To confirm the results further, instead of taking diffused light for the observations, direct sunlight was focussed on the instrument and the colour fringes were found much brighter than before, as expected. In this connection, on going through the special literature on the subject, a reference is found, though passingly, to the presence of these coloured fringes which gives strong support to our observation.¹ So much about the main principle underlying the qualitative examination of butter fat.

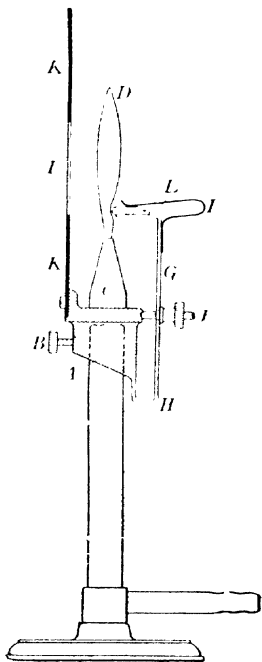
Special precautions have got to be taken in preparing the sample for being subjected to the examination. These are given below :

- (a) *Preparation of the sample for analysis* :—Melt the fat on a water bath and stir for uniform mixing. Transfer a small portion to a small Erlenmeyer flask and add a small quantity of pure anhydrous sodium sulphate for removing any trace of water that may be present. Filter this sample while hot through a filter paper. For qualitative test, a couple of grammes are more than sufficient.
- (b) *Preparation of the sample for second examination*: Another sample for examination should be prepared by thoroughly washing it with slightly warm water containing 2-5% alcohol in order to dissolve the water-soluble and alcohol soluble

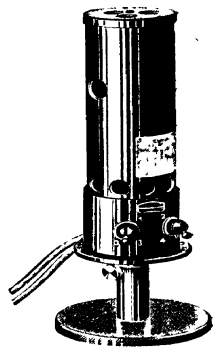
(1) Gruen : *Analyse der Fette und Wache*, Vol. I, p. 127; also K. Teichert.



The Butter Refractometer, with the water-jacketed double prism $P^1 P^2$; the box-shaped centre part D and the telescope F .
 Z - Inlet of the heating water,
 A - Outlet of the latter.



Sodium Burner



Electric Sodium Vapour Lamp on new stand.

rancidity-bearing constituents which may be present in the sample under examination. It is then freed from water, dehydrated and filtered. Another reading should be taken with this sample as before.

- (c) *Refractometric-Examination* : The prism and the metal surface should be carefully cleaned with a mixture of alcohol and ether (1 : 1) using a soft and clean chamois skin. Drop the sample under examination by means of a round-edged glass rod just enough to cover the prism surface. Key the two prisms together and take the refractometric reading after waiting for three minutes.

A constant temperature of 40°C. should be maintained throughout the investigation. Attempts made to take the readings at any other temperature and especially at lower temperatures and later to introduce a correction factor are likely to give erroneous results.¹

Air bubbles within the stratum of oil or fat must be avoided as they impair the distinctness of the critical line. Clear light should be properly focussed into the apparatus.

It is necessary that the adjustment of the instrument with the standard liquid supplied should be tested periodically. With careful handling of the apparatus, no variation in the adjustment takes place for a pretty long time. The standard liquid itself should be changed when ever it gets cloudy.

Finally, after the examination is over, the prism should be thoroughly cleaned with alcohol-ether mixture.

While observing the critical line of separation, '*Godbole-Sadgopal-lines*', the reading should be taken at the point

(1) Delaite : Bull. de l' Assoc. Belge. de Chimistes, 1894, 5, 145.
Analyst, 1895, 95.

where the dark field just separates either from the colourless portion of the field or from the first line of colour fringe. Always the reading should be taken with this precaution, otherwise the thickness of the colour fringe will make the readings vary within appreciable range.

General considerations of the Refractometric Examination : The refractive index of a pure substance under given conditions is a physical constant. Various glycerides possess different values of refractive index (accompanied with characteristic dispersion in the case of the Butyrorefractometer). The refractive indices of the various glycerides have been given by Lewkowitch.¹

Instead of using dispersed sunlight for the observations, when use was made of a monochromatic source of light (Sodium light), it was found, as expected, that the line of separation was sharper but without any colour fringe.

The magnitude of the refractive index is found to increase with the number of carbon atoms in the molecule of the fatty acid; the unsaturated fatty acids show a higher refractive index than the corresponding saturated acids.

Wollny provided the instrument with a thermometer which is in itself so graduated as to read the highest butter value allowable at the temperature at which the examination is being made. Another thermometer has been suggested by E. Bair,² which provides for the discrepancy created in the butter by the result of summer feeding. Using either forms of the special thermometer, the criterion of the purity of the butter-fat is, according to the authors, as follows.³ "If the telescope reads a higher number than

(1) Lewkowitsch, J. Chem. Tech. and Ana. of oils fats and waxes, ed. 5. vol. 1. p. 6-37 ;

(2) E. Bair. Zeit.f. Unters. d. Nahr. n. Genuss, 1902, p. 1145 ;

(3) Zeiss : The Butter Refractometer, e. 7. p. 6.

the thermometer, the sample is to be regarded with suspicion, otherwise it is pure." A sample of pure coconut oil (which gives a lower telescopic reading than that of pure butter-fat) should, according to the above authors, be pronounced as pure butter-fat. This is a discrepancy in the general statement made by Wollny and Bair.

Refractometric-cum-colour-fringe Examination of the Oils and Fats: In a previous note by the present authors it has been proved that the refractometric range for pure samples of butter-fats obtained from different sources and various parts of this country is limited between 40.0—43.5° at a temperature of 40°C and the colour fringe observed, though invariably colourless, is also at times violet tinged. That has also been stated in another publication on the subject¹. The results of the examination conducted with other oils and fats are given below :—

Table No. 21.
Vegetable Oils.

No.	Oil or Fat.	Refractometric value at 40°C.	Godbole-Sadgopal-Line.
1.	Cocogem ..	34.2	Deep Orange.
2.	Coconut Oil (Cochin) ..	34.25	" "
3.	Coconut Oil (Ceylon) ...	35.5	" "
4.	Kokum ..	45.6	" "
5.	Olive Oil ...	54.7	Bluish-Green.
6.	Almond Oil ...	58.0	" "
7.	Groundnut Oil ..	55.65	" "
8.	Mustard Oil ..	59.55	Yellowish-Green.
9.	Sesame Oil (Mixed seed) ..	59.0	Bluish-Green.
10.	Sesame Oil (White seed) ..	60.0	" "
11.	Sesame Oil (Black seed) ..	60.5	" "
12.	Linseed Oil ...	62.0	" "
13.	Poppyseed Oil ...	62.4	" "
14.	Safflower Oil ..	63.6	;; "
15.	Mahuwa Oil ..	63.0	" "
16.	Castor Oil ..	67.0	" "

(I) Butterfat : N. N. Godbole and Sadgopal,, Ed. 1930, p. 31.

Table No 22.

Animal Fats.

No.	Oil or Fat.	Refractometric value at 40°C.	Godbole-Sadgopal-Line.
1.	Candelite	40.0	Blue
2.	Talgol (at 50°C.)	44.0	"
3.	Mutton Tallow	45.5	"
4.	Beef Tallow	49.0	"
5.	Lard	50.55	"
6.	Horse Fat	54.0	"
	Goose Fat	50.5	"
8.	Tiger Fat	48.25 at 45°C.	Yellowish green.

Table No. 23.

Special Fats.

No.	Oil or Fat.	Refractometric value at 40°C.	Godbole-Sadgopal-Line.
1.	Vegetable Ghees	51.0-55.0	Blue
2.	Oleo-Margarine	48.6-49.2 ¹	Not examined as they were not available in India. }
3.	Margarine	50.3-58.2	

Table No. 24.

Free Fatty Acids.

No.	Name of the Acid.	Refractometric value at 40°C.	Godbole-Sadgopal Line.
1.	Oleic Acid	44.4	Bluish-green.
2.	Palmitic Acid	49.0	Blue.
3.	Capric Acid	7.0	Orange.
4.	Lauric Acid	14.0	Orange.
5.	Linolic Acid	78.0	Bluish-green.
6.	Ricinolic Acid	57.5	" "

(I) G. Baumart, Zeit-Schrift für Unter-Suchung der Nahrungs und Genuss-mittel, vol. 9, 134, 1905.

From the above tables, it is clear that without exception the individual oils and fats give their characteristic fringes depending upon the glycerides of which they are composed. A number of qualitative mixtures have been tested and found to agree with the general observation. Even in such a case the use of direct sunlight or light from a Mercury-arc-lamp gives pronounced results. Below are given the results of some very judicious mixtures :—

Table No. 25.

S. number.	Nature of the components of the mixture.	Refractometric value at 40°C.	Godbole-Sadgopal-Line in	
			Diffused Sunlight.	Arc-Light.
1	Lard + Ghee + Coconut Oil ..	43.5	Blue + light Orange,	Blue + clear orange
2	Tallow + Ghee + Coconut Oil ..	44.0	"	"
3	Vegetable ghee + Ghee + Coconut Oil.	42.4	"	"
4	Tallow 10% + Ghee 75% + Paraffin Wax 5% + Coconut Oil 10%	41.0	"	"

The last sample is of special interest as it was given for an examination to some students in this Laboratory and the general values were as follows :

A-Value	6.3 ;
B-Value	26.3 ;
Saponification Value	222.3 ;
Iodine Value	25.17
Reichert Meissl Value	25.63 ;
Reichert Polenske Value	3.3 ;

It was only because of the observation of Godbole-Sadgopal-line that the adulteration could be easily detected. This experiment should prove the usefulness of the method for practical purposes.

The results obtained regarding the colour fringes have been noted with very great clearness when seen under the light emitted out by a mercury vapour lamp. For the observations given below, a lamp designed and presented by Sir C. V. Raman was used :—

Table No. 26.

S. num-ber.	Oil or Fat or fatty acid.	Godbole-Sadgopal Line.	Nature of the band.
1	Cocogem ..	Light Orange ..	Thin band but scattered light.
2	Coconut Oil ..	" ..	"
3	Caproic Acid ...	" ..	"
4	Mahuwa oil ..	Bluish Violet ..	Thin band
5	Lard ...	" ..	"
6	Oleic Acid ...	" ..	"
7	Talgol ..	" ..	Medium band
8	Vegetable Ghee ...	" ..	Thin Band,
9	Linolic Acid ..	Greenish Blue ...	"
10	Safflower Oil ..	Deep Violet-Blue ..	Broad Band,
11	Groundnut Oil ..	" ..	"
12	Olive Oil ...	" ..	"
13	Black Sesame Oil ..	" ..	"
14	White Sesame Oil ..	" ..	"
15	Linseed Oil ...	" ..	"
16	Castor Oil ..	" ..	"
17	Poppy-seed Oil ..	" ..	"
18	Almond Oil ...	" ..	"
19	Mustard Oil ..	" ..	"
20	Ricinolic Acid ..	" ..	"
21	Butter-fat ..	Colourless ..	Thin Band..

An attempt was made to quantitatively measure the dispersion of various oils and fats by means of Abbe's Refractometer. The various colour-fringes as observed in the case of Butyro-Refractometer were no doubt observable in this case also, of course, with some additional characteristics. But an attempt to measure the dispersion of individual oil or fat could not show such an appreciable difference as to warrant its application for the purpose of detecting adulteration in butter-fat.

Another interesting observation with regard to the Refractometric examination of the samples of butter fats under examination is that in cases of adulterants with different solidifying points, separation can be made by quickly cooling the sample under examination in a freezing mixture. Two distinct layers separate in such cases which give entirely different readings with the instrument and also very different "Godbole-Sadgopal-Lines". This observation is of very great importance in sorting the samples correctly.

It is our desire that every worker on this subject should add one additional column in the investigation of ghee and note his results about "Godbole-Sadgopal Lines". There is no doubt that at least in a majority of cases in India, the adulteration of butterfat could be easily ascertained by this method. Our observations regarding these colour fringes in the case of adulterated samples of butterfat are being tested and verified in many Indian laboratories. The following remarks from the Public Analyst of Calcutta Corporation, one of the most progressive municipalities in India, substantiate our inferences :

· "Your investigation of the coloured fringes observed in the Butyro-refractometer has been closely followed by us. It is really helpful as a qualitative test in detecting adulteration in ghee. Even when the percentage of adulteration is so low as not to appreciably increase the index, a trained eye will easily be able to differentiate between the red-violet band of pure ghee and *bluish-purple* band of adulterated samples.

While carrying on investigations on titre test of pure cow and buffalo ghee, I have observed that the insoluble fatty acids also show similar coloured fringes at the critical line. B. R. index of insoluble fatty acids of cow and buffalo ghee vary within the limits of 29.8 to 31.0 at 40° C. with clear red-violet band while those of vegetable oils vary within 38.0 to 40.0 at the same temperature with a blue band".

Our observations with regard to the reliable application of 'Godbole-Sadgopal Lines' in Butyro-refractometer have been further substantiated by the work of Athawale and Jatkar¹ with Pulfrich-refractometer (For details, see page 63 of this book).

(1) Journal of the Indian Institute of Science, Vol. 21A, part 3, pp. 1-25.

CHAPTER IX

Table No 27 for the Conversion of Butyro Refractive Readings into Corresponding Refractive Indices.

4th decimal place of n_D .											
n_D .	0	1	2	3	4	5	6	7	8	9	
Scale readings of Butter-refractometers.											
1.417	5.0
1.418	..	.9	4.8	4.7	4.5	4.4	4.3	4.2	4.1	3.9	3.8
1.419	..	3.7	3.6	3.5	3.3	3.2	3.1	3.0	2.8	2.7	2.6
1.420	..	2.5	2.3	2.2	2.1	2.0	1.9	1.7	1.6	1.5	1.4
1.421	..	1.2	1.1	1.0	0.9	0.8	0.6	0.5	0.4	0.2	0.1
Scale Central Scale-readings.											
Scale -reading	0	1	2	3	4	5	6	7	8	9	*
1.422	0	0	1	2	4	5	6	7	9	*	*1
1.423	1	2	4	5	6	7	9	*0	*1	*2	*4
1.424	2	5	6	7	8	*0	*1	*2	*3	*5	*6
1.425	3	7	8	*0	*1	*2	*3	*5	*6	*7	*8
1.426	5	0	1	2	*4	*5	*6	*7	*9	*0	*1
1.427	6	2	4	5	6	8	9	*0	*1	*2	*4
1.428	7	5	6	7	9	*0	*1	*2	*4	*5	*6
1.429	8	7	9	*0	*1	*2	*4	*5	*6	*8	*9
1.430	10	0	1	.3	.4	.5	.6	.7	.9	*0	*1
1.431	11	3	4	5	6	8	9	*0	*2	*3	*4
1.432	12	5	7	8	9	*1	*2	*3	*5	*6	*7
1.433	13	8	*0	*1	*2	*4	*5	*6	*7	*9	**9
1.434	15	1	3	.4	.5	.6	.8	.9	*0	*2	*3
1.435	16	4	6	7	8	0	*1	*2	*4	*5	*6
1.436	17	8	*9	*0	*2	*3	*4	*5	*7	*8	*9
1.437	19	1	2	.3	.5	.6	.7	.8	*0	*1	*3
1.438	20	4	5	6	8	9	*1	*2	*3	*4	*6
1.439	21	7	8	*0	*1	*2	*4	*5	*6	*7	*
1.440	23	0	2	.3	.4	.5	.7	.8	.9	*1	*2

Table No. 27 (contd.)

1.441	24	3	5	6	7	3	*0	*1	*2	*4	*5
1.442	25	6	8	9	*1	*2	*3	*5	*6	*7	*9
1.443	27	0	1	3	4	.5	.7	.8	.9	*1	*2
1.444	28	3	5	6	7	9	*0	*2	*3	*4	*6
1.445	29	7	9	*0	*1	*3	*4	*7	*7	*8	*9
1.446	31	1	2	.4	.5	.6	.8	.9	*1	*2	*3
1.447	32	5	6	8	9	*0	*2	*3	*5	*6	*7
1.448	33	9	*0	*2	*3	*4	*6	*7	*9	**0	**1
1.449	35	3	.4	.6	.7	.8	*0	*1	*3	*4	*5
<hr/>											
1.450	36	7	8	*0	*1	*2	*4	*5	*7	8*	*9
<hr/>											
1.451	38	1	2	.3	.5	.6	.7	.9	*0	*2	*3
1.452	39	5	6	7	9	*0	*1	*3	*4	*6	*7
1.453	40	9	*0	*1	*3	*4	*5	*7	*8	**0	**1
1.454	42	3	.4	.5	.7	.8	*0	*1	*3	*4	*6
1.455	43	7	9	*0	*2	*3	*4	*6	*7	*9	**0
1.456	45	2	3	.5	.6	.7	*9	*0	*2	*3	*4
1.457	46	6	7	9	*0	*2	*3	*5	*6	*7	*9
1.458	48	0	2	3	.5	.6	.8	.9	*1	*2	*4
1.459	49	5	7	8	*0	*1	*2	*4	*5	*7	*8
<hr/>											
1.460	51	0	1	3	.4	.6	.7	.9	*0	*2	*3
<hr/>											
1.461	52	5	7	8	*0	*1	*3	*4	*6	*7	*0
1.462	54	0	2	3	.5	.6	.8	*0	*1	*3	*4
1.463	55	6	7	9	*0	*2	*3	*5	*6	*8	*9
1.464	57	1	3	4	.6	.7	.9	*0	*2	*3	*5
1.465	58	6	8	9	*1	*2	*4	*5	*7	*8	**0
1.466	60	2	3	5	.6	.8	.9	*1	*2	*4	*5
1.467	61	7	8	*0	*2	*3	*5	*6	*8	*9	**1
1.468	63	2	4	.5	.7	.8	*0	*2	*3	*5	*7
1.469	64	8	*0	*1	*3	*4	*6	*7	*9	**1	**2
<hr/>											
1.470	66	4	5	.7	.8	*0	*2	*3	*5	*7	*8
<hr/>											
1.471	68	0	1	2	4	6	7	9	*1	*2	*4
1.472	69	5	7	9	*0	*2	*3	*5	*7	*8	**0
1.473	71	1	3	4	.6	8	.9	*1	*2	*4	*5
1.474	72	7	9	*0	*2	*3	*5	*7	*8	**0	**1
1.475	74	3	5	.6	.8	*0	*1	*3	*5	*6	*8
1.476	76	0	1	3	5	.7	.8	*0	*2	*3	*5

Table No. 27 (contd.)

1.477	77	7	9	*1	*2	*4	*6	*7	*9	**1	**2
1.478	79	4	6	.8	*0	*1	*3	*5	*6	*8	**0
1.479	81	2	3	5	.7	.9	*0	*2	*4	*5	*7
1.480	82	9	*1	*2	*4	*6	*8	*9	**1	**8	**5
1.481	84	6	8	*0	*2	*3	*5	*7	*9	**0	**2
1.482	86	4	6	.7	9	*1	*3	*5	*6	*8	**0
1.483	88	2	3	5	7	.9	*1	*2	*4	*6	*8
1.484	90	0	2	3	5	7	.9	*1	*2	*4	*6
1.485	91	8	*0	*1	*3	*5	*7	*9	**0	**2	**4
1.486	93	6	.8	*0	*1	*3	*5	*7	*9	**1	**2
1.487	95	4	6	.8	*0	*1	*3	*5	*7	*9	**0
1.488	97	2	4	6	.8	*	*1	*3	*5	*7	*9
1.489	99	1	2	4	6	.8	*0	*2	*3	*5	*7
1.490	100	9	*1	*3	*4	*6	*8	**0	**2	**4	**3
1.491	102	7	9	*1	*3	*5	*6	*8	**0	**2	**4
1.492	104	8	8	105.0	.—	.—	.—	.—	.—	.—	.—

(From a pamphlet published by Messrs Carl-Zeiss of Jena.)

CHAPTER X

Quantitative Estimation of the Adulteration in Butterfat. (Method of Bertram, Bos and Verhagen.)

The quantitative determination of the adulteration of butter fat is by no means an easy or simple problem. Excepting the method described below, no other method is known which can approach it in point of accuracy or general manipulation. The method is based on the following line of reasoning.

The most important and the least variable constituent of butter fat is its content of butyric glyceride. The variation in the percentage of butyric acid available in butter fat is very small, *i. e.*, roughly speaking 2.7 to 3.6 per cent. (B-value being 33 to 35.5). The estimation of this particular glyceride, therefore, from an analytical point of view is an extremely important factor. There is no known adulterant of butter fat which contains the glyceride of this acid in any appreciable amount, *i. e.*, the adulterant will naturally reduce the percentage of available butyric-glyceride in a sample of ghee under examination. Even in the case of human-milk-fat, butyric acid is reported to be absent or nearly so¹. Indeed, it may be noted in this connection that just for this very reason attempts are being made at manufacturing the glyceride of butyric acid, its esters and its salts synthetically², (along with the other glycerides of odd number of carbon atoms)

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1. Elsdon : Edible Oils and Fats, P. 20 ;
 2. D. Holde. Kohlenwasserstoffe u. Fette, 1924, p. 621; Ad. Gruen, Analyse der Fette u. Wachse, Vol. I, ed. 1925, p. 171.

and if this is achieved. we are afraid the question of the analysis of ghee will have lost, perhaps, the last leg on which it stands to-day.

The range of the A and B values¹ of European samples of butter-fat has been found to be very strikingly in agreement with that of Indian butter-fats, established by an extensive series of experiments carried out by the present authors. The results obtained in this connection by Professor Prakash Chandra, M. Sc., of Punjab Education Service who worked here on some characteristic samples again confirm the same values given in the beginning of this paper. All these facts surely go to enhance the value of the applicability of this method. It has been stated above that the general adulterants of butter fat are very poor in both their A and B values (except in the case of coconut oil and palmkernel oil which have got a very high A value) and to confirm this, certain typical adulterants like vegetable ghee, coconut-oil. and mutton tallow etc., were selected and examined. The results obtained are tabulated below.

Table No. 28
A and B values of Adulterants.

S. num ber.	Name.	A-value.	B-value.
1	Vegetable Ghee (Chakra Brand)	0	0.38
2	Vegetable Ghee (Laxmi Brand)	0.09	0
3	Cocogem	23.65	2.53
4	Coconut-Oil	26.9	2.43
5	Mutton Tallow	0.88	0.52
6	Mahuwa Oil	0.67	1.16
7	Sesame Oil	0.5	0.6

1. Bertram, Bos and Verhagen. 'Chem. Weekblad, 1923, 20, 610.

It should be made clear here that in pronouncing an opinion on the purity of a sample under examination, both the A and B values must be taken into account; of the two values, the B value is more important. The change in the A value is not very great, in certain cases of adulteration, but the corresponding change in the B value is enough to detect the adulteration. The results in this connection are given below both in the form of a table and in the form of a curve.

Table No. 29.

A and B values obtained with mixtures of Butter-fat and Vegetable ghee.

S. No.	Oil or Fat.	A-Value.	B-Value.
A	100 % Butter-Fat.	6.48	33.01
B	100 % Vegetable Ghee.	0	0.9
1	1% " 	6.4	32.65
2	2% " 	6.35	32.25
3	3% " 	6.3	32.0
4	4% " 	6.2	31.7
5	5% " 	6.1	31.4

Having satisfied ourselves with the accuracy and the reliability of the A and B values and also the colour fringes, it was thought advisable to try some definite mixtures of butter fat adulterated with (a) coconut oil, (b) vegetable ghee, (c) Mutton tallow, (d) Mahuwa oil, and (e) Sesame oil, both qualitatively and quantitatively. The results are tabulated in the following tables which have been further graphed into their corresponding curves. It should be recorded here that the curve of a mixture of butter fat and coconut oil as obtained here fully tallies with the curve

given by Bertram, Bos and Verhagen¹ to which reference has already been made. The other curves for mixtures of vegetable ghee and mutton tallow etc., with butter-fat are worked in this laboratory.

Table No. 30.

Examination of Mixtures of Butter Fat and Coconut Oil.

See Curve No. 1.

No.	Percentage of Coconut Oil.	A-Value.	B-Value.	Refractometric Value.	Godbole-Sadgopal-Line.
1	0.5%	6.9	32.95	42.15	Light-Orange
2	1.0%	7.0	32.9	42.05	" "
3	1.5%	7.2	32.4	42.0	" "
4	2.0%	7.5	32.2	41.5	" "
5	2.5%	7.8	32.0	41.5	" "
6	5.0%	8.0	31.5	41.0	Orange
7	7.5%	8.75	31.0	40.5	"
8	10.0%	9.0	30.5	40.5	"
9	12.5%	9.5	29.75	40.5	Dark-Orange
10	15.0%	10.0	29.25	40.2	"
11	17.5%	10.5	28.75	40.15	"
12	20.0%	11.0	28.0	40.15	"
13	25.0%	12.5	26.5	40.15	Deep-Orange
14	30.0%	13.75	25.0	40.1	" "
15	35.0%	15.0	23.5	40.0	" "
16	40.0%	16.75	22.0	39.3	" "
17	45.0%	17.75	20.5	38.85	" "
18	50.0%	19.25	19.0	38.75	" "
A	Butter Fat	6.48	33.01	42.2	Light Violet
B	Coconut Oil	26.9	2.43	35.4	Deep Orange.

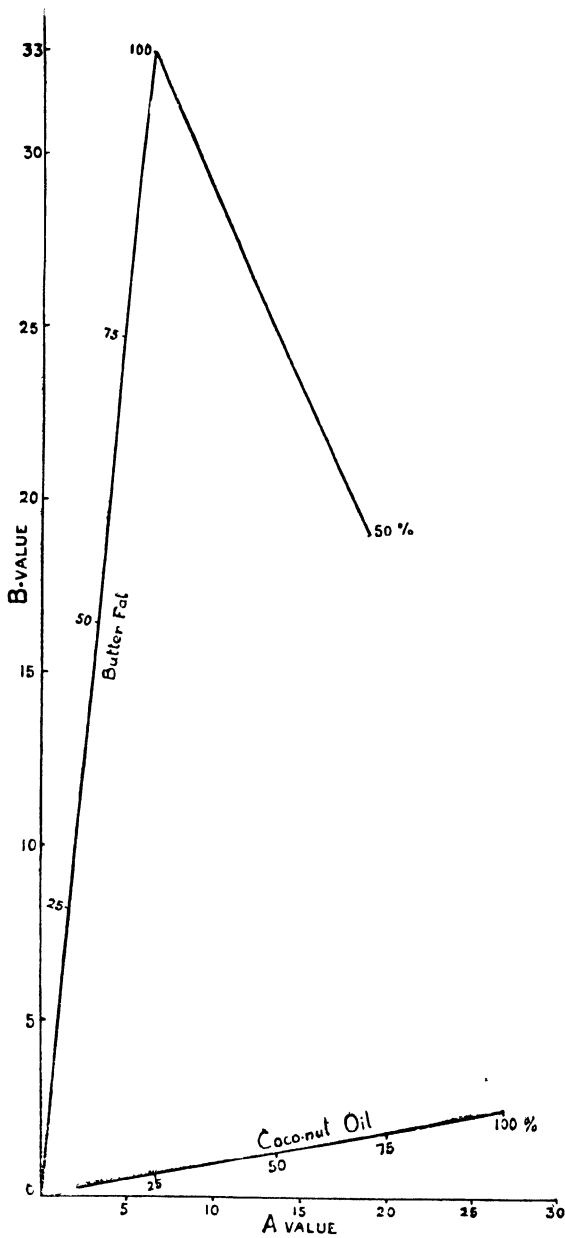
Table No. 31.

Examination of Butter Fat Adulterated with Vegetable Ghee.

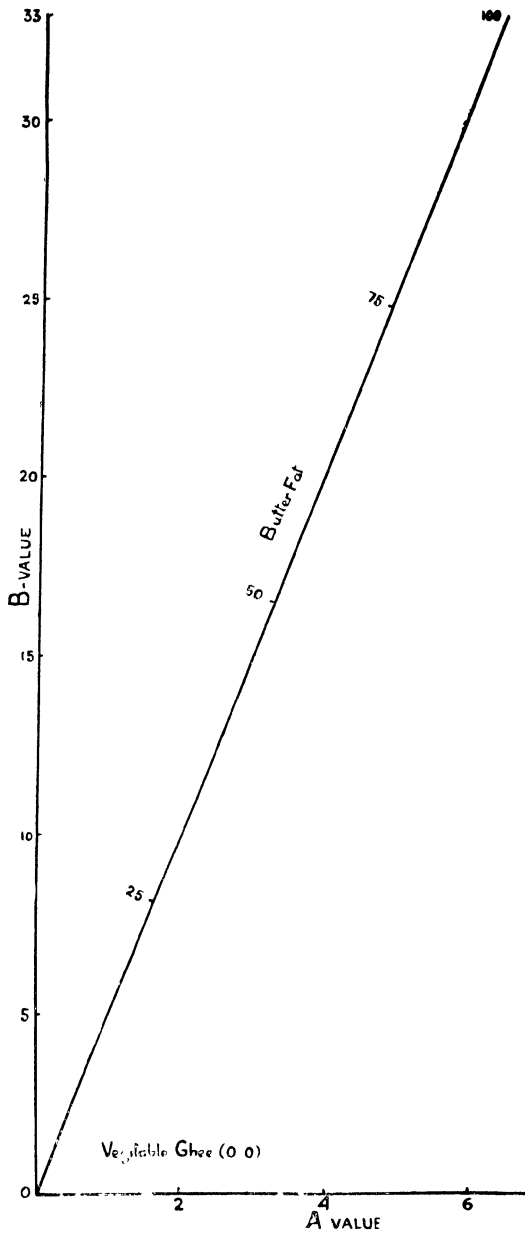
See Curve No. 2.

No.	Percentage of Vegetable Ghee.	A-Value.	B-Value.	Refractometric value.	Godbole-Sadgopal Line.	Calculated percentage of Vegetable Ghee.
1	0.5%	42.6	Light Blue	
2	1.0%	42.72	" "	
3	2.0%	43.0	" "	
4	2.5%	43.0	" "	
5	5.0%	6.15	31.4	43.05	Blue	4.85%
6	10.0%	5.7	29.75	43.15	"	9.9%
7	15.0%	5.5	28.0	43.5	"	15.15%
8	20.0%	5.2	26.75	44.0	"	18.95%
9	25.0%	4.9	24.75	44.4	Deep Blue	25.0%

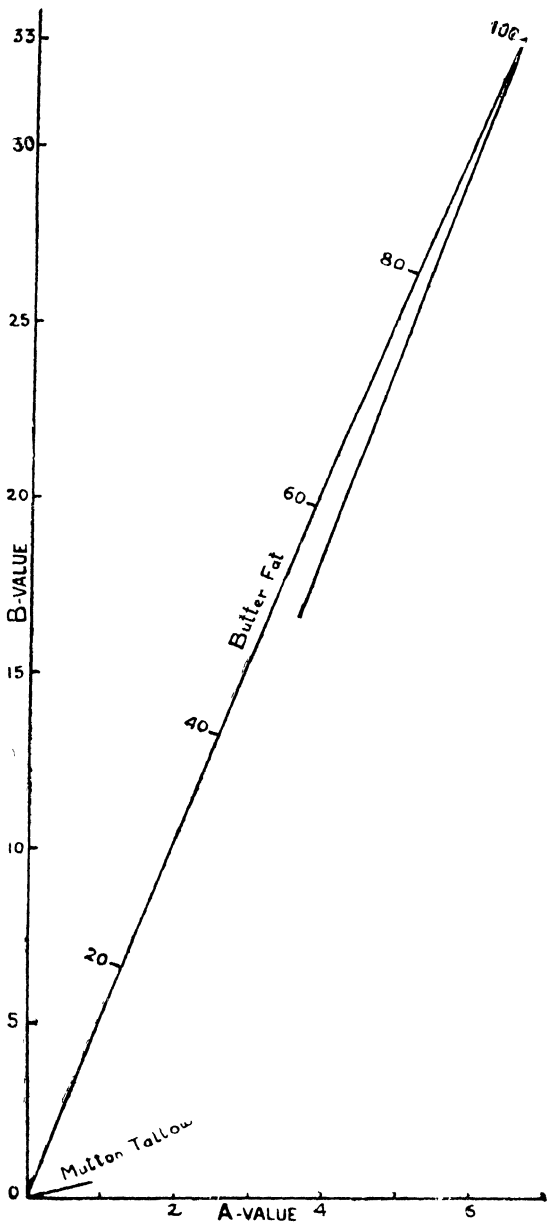
1. Bertram, Bos & Verhagen; Chem. Weekblad, 1923, 20, 610. (contd.)



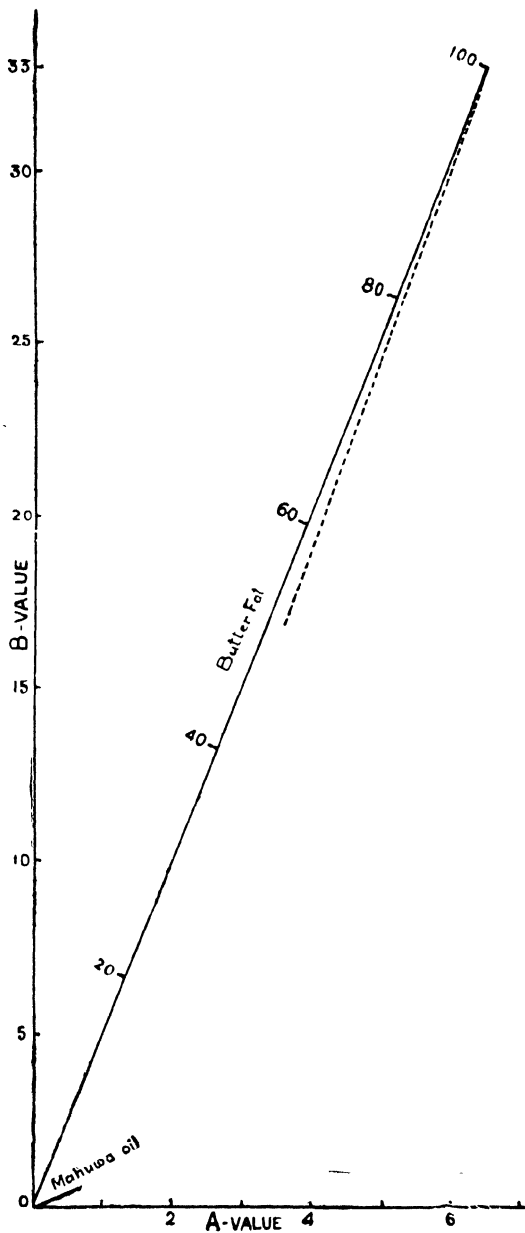
(1) Butterfat adulterated with Coconut Oil.



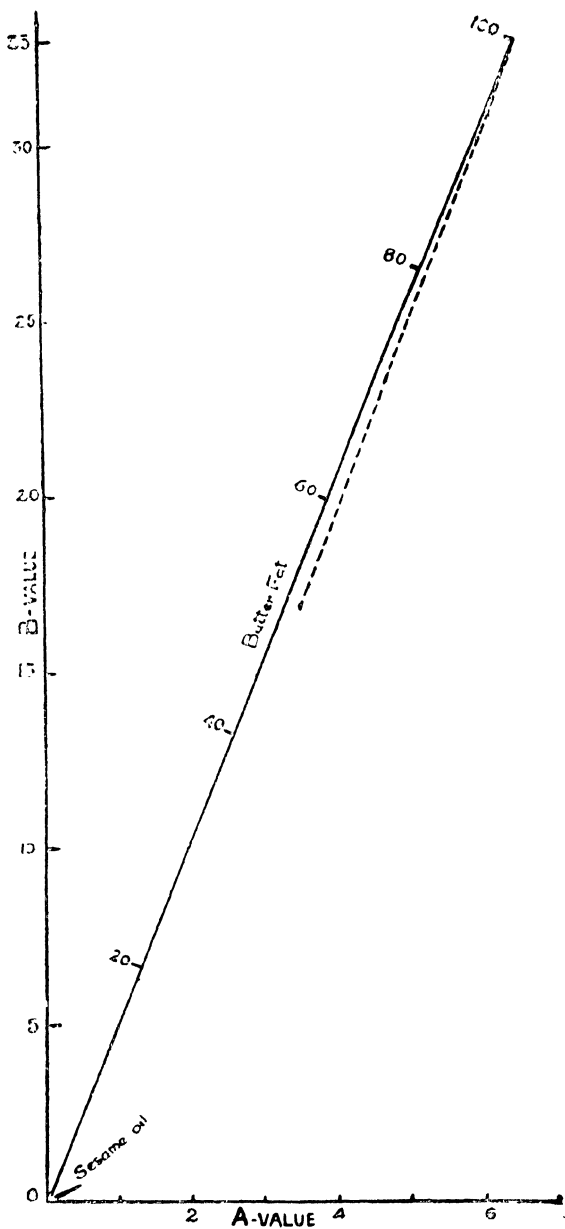
(2) Butterfat adulterated with Vegetable Ghee.



(3) Butterfat adulterated with Mutton Tallow.



(4) Butterfat adulterated with Mahuwa Oil



(5) Butterfat adulterated with Sesame oil.

Table No. 31.—(contd.)

No.	Percentage of Vegetable Ghee.	A-Value.	B-Value.	Refractometric value.	Godbole-Sadgopal Line.	Calculated percentage of Vegetable Ghee.
10	30.0%	4.5	23.0	45.0	" "	30.0%
11	35.0%	4.2	21.75	45.3	" "	34.1%
12	40.0%	3.9	19.75	45.7	" "	39.85%
13	45.0%	3.5	18.0	46.25	" "	45.46%
14	50.0%	3.25	16.5	46.75	" "	50.0%
A	Butter-fat	6.5	33.03	42.0	Light-violet	100%
B	Vegetable Ghee	0	0.09	52.5	Blue	Butter-fat. 100% Veg. Ghee.

Table No. 32.

Examination of Butter-fat Adulterated with Mutton Tallow.

See Curve No. 3.

Serial No.	Percentage of Tallow.	A-Value.	B-Value.	Refractometric value.	Godbole-Sadgopal Line.	Calculated percentage of tallow.
1	0.5%	42.1	Light Blue	
2	1.0%	42.15	"	
3	2.0%	42.4	"	
4	2.5%	42.4	"	
5	5.0%	6.2	31.4	42.45	Bluish green	4.85%
6	10.0%	5.9	29.75	42.5	"	9.9%
7	15.0%	5.7	28.0	42.55	"	15.1%
8	20.0%	5.4	26.5	42.7	"	19.7%
9	25.0%	5.0	24.5	43.0	Blue	25.79%
10	30.0%	4.7	23.0	43.1	"	30.0%
11	35.0%	4.5	21.75	43.4	"	34.1%
12	40.0%	4.2	19.8	43.6	"	40.0%
13	45.0%	4.0	18.5	43.6	"	44.0%
14	50.0%	3.65	16.5	44.0	"	50.0%
A	Butter-fat	6.5	33.03	42.0	Light violet	100%
B	Mutton Tallow	0.88	0.52	45.5	Greenish blue	Butter-fat 100% Tallow

Table No. 33.

Examination of Butter-fat Adulterated with Mahuwa Oil.

See Curve No. 4.

Serial No.	Percentage of Mahuwa Oil.	A-value.	B-value.	Refractometric value.	Godbole-Sadgopal Line.	Calculated percentage of Mahuwa Oil.
1	2.0%	43.0	Light Blue	
2	5.0%	6.5	31.26	44.25	Light Bluish-green.	4.8

Serial No.	Percentage of Mahuwa Oil.	A-Value.	B-Value.	Refractometric value.	Godbole-Sadgopal Line.	Calculated percentage of Mahuwa Oil.
3	10.0%	5.5	29.7	45.5	Bluish green	9.75
4	15.0%	5.25	28.0	46.0	"	15.05
5	20.0%	4.9	26.0	46.4	"	19.0
6	25.0%	4.5	24.75	47.0	"	25.15
7	30.0%	4.2	23.0	47.75	"	30.0
8	35.0%	4.0	21.75	48.5	"	34.2
9	40.0%	3.75	19.5	50.05	"	39.75
10	45.0%	3.3	17.75	52.25	"	45.2
11	50.0%	3.25	16.5	54.0	"	50.0
A	Butter-fat	6.5	33	42.0	Colourless	100% Butter-fat
B	Mahuwa	0.65	0.6	63.0	Bluish green	100% Mahuwa Oil.

Table No. 34.

Examination of Butter-fat Adulterated with Sesame Oil.

See Curve No. 5.

Serial No.	Percentage of Sesame Oil.	A-value	B-value	Refractometric value.	Godbole-Sadgopal Line.	Calculated percentage of Sesame Oil.
1	2.0%	44.5	Light Blue	..
3	5.0%	6.0	31.0	45.0	Light Bluish green.	4.9
3	10.0%	5.8	29.5	45.25	Bluish Green	9.9
4	15.0%	5.45	28.0	45.7	"	14.9
5	20.0%	5.1	26.5	46.3	"	19.2
6	25.0%	4.8	24.5	47.0	"	24.85
7	30.0%	4.4	23.0	47.5	"	30.1
8	35.0%	4.15	21.5	48.25	"	34.5
9	40.0%	4.0	19.0	49.5	"	39.75
10	45.0%	3.5	17.75	51.2	"	45.0
11	50.0%	3.0	16.0	52.5	"	50.0
A	Butter-fat	6.5	33.0	42.0	Colourless	100% Butter-fat.
B	Sesame Oil	0.5	0.6	60.0	Bluish Green	100% Sesame Oil.

A study of the above tables and experimental data contained therein goes to prove the usefulness of the methods proposed beyond any doubt. A very serious position arises when some synthetic chemical compounds like fatty acids, or fatty acid-glycerides or similar addi-

tions are made to increase the B value favourably. In all such cases of suspicion or if very high acid value is obtained, it is necessary to give a preliminary treatment to the fat under examination before actually putting it for B value determination. The treatment, as has been already explained, consists in washing the fat with warm water for some time and then decanting off the pure fat separately. A number of such treatments may be resorted to in special cases and thus the fat is rendered free from all such additions which are not really part of the fat itself but are added externally for fraudulent purposes. In case of rancid samples, this is all the more necessary. All these compounds being water-soluble are very easily removable. A very weak solution of alcohol in water may also be used but not absolute alcohol as it dissolves some of the water-soluble lower glycerides originally present in the fat.

In this connection the following note re. the "Detection and Determination of Diacetyl in Butter" by W. L. Davies¹ will be found of much interest and, therefore, is given below :—

"To detect traces of diacetyl in butter, the di-ketone may be condensed with hydroxylamine to form dimethylglyoxime, which yields a red nickel compound. Also, the Voges-Proskaner reaction may be used ; *viz.* in strong alkaline solution diacetyl gives a deep red colour with various compounds containing a guanidine nucleus, such as peptone solution or creatine. As little as 1/100 mgr. of diacetyl may thus be detected but the depth of colour is proportional to the amount present. To determine the diacetyl, the nickel glyoxime method is used. From 0.5 to 1 kilo. of butter is distilled in the presence of 500 ml.

(1) Food Manufacture, 1933, 346-348.

of a mixture of 0.1 N sulphuric acid and one per cent acetic acid, and a drop of oleic acid is added to oxidise any carbinol to diacetyl. The mixture is heated for 20 minutes in an all-glass apparatus beneath a reflux condenser, and 40 ml. of distillate are then collected and kept at 90°C. for 1 hour after being mixed with 10 ml. of a buffered nickle reagent (1 part of 20 per cent hydroxylamine hydrochloride, 1 part of 10 per cent nickel sulphate solution, 2 parts of 20 per cent sodium acetate solution.) After the liquid has been cooled and neutralised to PH. of 7.2, the nickel compound is left to settle for 24 hours; the liquid is then filtered through a tared sintered Jena crucible (IG₃), the precipitate is dried at 110°C. and weighed, and the weight is multiplied by 0.596 to obtain the weight of diacetyl. Repeated determinations of known amounts of diacetyl gave results with 97 to 103 per cent of theory. If the diacetyl is to be estimated from the intensity of the red colour of Gooch filter discs,¹ 40 to 100 grams. of butter and 100 ml. of water are directly distilled, and no neutralisation is necessary."

It will be interesting to find out the effect of the oil insoluble colours and chlorophyll on the "*Godbole. Sadgopal-Lines.*" Another important modification, which requires thorough trial before announcement, is to find out whether it is possible or not to estimate the A and B values by proceeding with a smaller quantity of fat under examination and proportionately small amounts of the reagents. Work is proceeding in these two directions.

Conclusion.

In summarising the results, it is needless to point out that close attention has been given to examine all the known methods of physical and chemical analysis of butter

(1), Analyst, 1932, 57, 389.

fat. What is generally desired in such cases is the evolution of a dependable method of universal application. Consequently the two methods proposed are as follows.—

(i) *For Qualitative Examination:* The reading of Butyro-refractive Index and the observation of “Godbole-Sodgopal-Lines” in ordinary day light, or if available in presence of a Mercury-Arc-Lamp.

(ii) *For Quantitative Examination:* A correct estimation of A and B values.

Finally the results may be confirmed, if necessary, by determining Reichert-Meissl and Reichert-Polenske values along with the corresponding saponification values. As a routine, the two proposed methods are very satisfactory, handy, accurate and reliable.

CHAPTER XI

ESTIMATION OF A AND B VALUES.*

Detailed method of S. H. Bertram, H. G. Bos and F. Verhagen* as modified by the present authors.

(Reprinted from the Indian Soap Journal, Calcutta.)

Definition

The **A Value** is a measure for the content of fatty acids in an oil or a fat (or a mixture of the two) which give rise to water-soluble magnesium salts and water-insoluble silver salts of saturated acids with six, eight and ten carbon atoms and is, therefore, a measure for the content of those fatty acids which are characteristic of coconut, palmkernel and similar oils.

The **B Value** is a measure for the content of butyric acid, (a constituent found only in butterfat), whose magnesium and silver salts are soluble in water and also in a solution saturated with silver sulphate and is, therefore, useful for the estimation of butterfat.

Object

The object of the estimation of A-and B-Values* is, therefore, to estimate the purity and content of coconut oil, palmkernel oil and butterfat either individually or in mixtures.

Principle

The combination of the two operations adopted in the estimation of A and B Values covers the experimental data involved in the estimation of the Kirschner-Value† and also*

* Ch. Weekblad, Bd, 20, S. 610, 1923.

† Z. Nahrgsm. Bd. 9, S. 65, 1905.

the Magnesium-Value of Ewer. †† Moreover the estimation of A and B Values is proved to be simpler in execution and is much more reliable in results than the other two values.

The method is based on the following principle: The oil or fat under investigation, single or mixed, is saponified and with the help of magnesium sulphate solution, the insoluble magnesium salts of the fatty acids of high molecular weights are separated (from about C₁₂ onwards). The soluble magnesium salts of the lower fatty acids are treated with silver nitrate solution whereby the soluble silver-butyrate is separated from the other insoluble silver salts of the remaining acids in the presence of an excess of silver sulphate.

Preparation of the Sample

The oil or fat under examination should be thoroughly washed with distilled water in order to remove (a) any traces of chlorides which may be present as common salt or in any other form, (b) rancidity-bearing free fatty acids and esters etc. and (c) any other adulterants like Amyl-acetate or artificial Butteraroma which might influence the B value appreciably. The oil or fat should be separated from the water-layer, dehydrated with anhydrous sodium sulphate and filtered clear through a hot water-funnel.

Preliminary Procedure for the Separation of the Magnesium Salts of the higher

Fatty Acids

Exactly 25 gms. of the fat under examination are taken in a 300 C. C. Earlenmeyer flask and are saponified on a direct flame with 40 C. C. of glycerol potash solution (3 vols. of chlorine-free glycerine and 1 vol. of caustic potash solution made by dissolving 750 gms. of chlorine-

†† Z. Nahrgm. Bd. 19. S. 529, 1910.

free caustic potash in one litre of the solution) until no more froth is observed and a soap solution without any over-heating is obtained. This perfectly saponified solution is then allowed to cool and to this, 100 C. C. of distilled water are added. The whole of the solution is then transferred quantitatively to a 500 C. C. measuring flask and is thoroughly shaken before making it to the desired volume of 500 C. C. Then 400 C. C. of this solution are pipetted out in a 750 C. C. Earlenmeyer flask which is further heated to a temperature of 80°C. on a water bath. For this purpose, the flask is provided with a cork having two holes, one for the thermometer and the other to allow the vapours to escape. In the meanwhile, a solution of magnesium sulphate (prepared by dissolving 150 gms. of chemically pure magnesium sulphate in a litre of distilled water) is also heated to a temperature of 80°C. and 100 C. C. of this hot magnesium sulphate solution are added in portions with gradual shaking to the hot soap solution. The whole operation should be completed within five minutes. *For the purpose of such regulated addition, the pipette should be provided with a good tap at its delivery-end.* The control of the temperature as stated above is necessary to obtain a precipitate which can be very easily filtered clear. The precipitated mixture is then shaken for about ten minutes at the same temperature and is then cooled down to 20°-30°C. *in a bath for about 15 minutes.* It is again shaken thoroughly. The cooled precipitate is filtered on a large Gooch-filter (*previously cleaned with chromic acid and distilled water*) fitted with a suction arrangement.

Let this filtrate be called "F". This filtrate approximately amounts to 410 c. c.

Estimation of A-Value

200 C. C. of the filtrate "F" are then transferred to a 250 C. C. measuring flask and are neutralised with a small quantity of semi-normal sulphuric acid solution using a 1% solution of phenolphthalein as indicator. Then 20 gms. of chemically pure sodium nitrate (free from chlorine) and 22.5 C.C. of N/5 chemically pure silver nitrate solution are added to the above. The whole mixture is shaken thoroughly. The solution is then made up to the volume of 250 C.C. with distilled water, shaken for another five minutes and then allowed to remain in water for about an hour at a temperature of about 20°-30°C. It is then filtered. To 200 C.C. of this filtrate, are added 6 C.C. of cold saturated solution of iron-alum (Ferric-ammonium-sulphate) and 4 C.C. of 40% nitric acid. Then the excess of silver nitrate is titrated with a decinormal solution of Ammonium sulphocyanide according to Vollar's method *based on the following principle :—

"Standardisation of the solution of ammonium-sulphocyanide : Place 20 C.C. of the decinormal solution of silver nitrate in a beaker, dilute it with water to about 100 C.C. and add a few C.C. of dilute nitric acid ; 1 C.C. of iron-alum solution is then added to serve as an indicator and the sulphocyanide solution is allowed to flow in from a burette with constant stirring until the red colouration of the liquid becomes permanent. From the volume of the solution required, its strength is then calculated according to the usual procedure of volumetric analysis."

Exactly in a similar way, a blank experiment should be carried out.

* Treadwell and Hall's Analytical Chemistry, Vol. 2 1924, pp. 601 602.

For neutralising the alkali in the blank experiment, it is desirable to use an acid stronger than N/2 because if the titration is done with a semi-normal acid as in the main experiment, the total volume of the mixture will exceed the volume of 250 c. c. as the total alkali present will require approximately 130 c. c. of N/2 sulphuric acid solution. If the reagents employed are pure, there should be no turbidity in the case of the blank experiment with the addition of the silver nitrate solution and for the final titration, about 36 c. c. of decinormal ammonium sulphocyanide solution should suffice.

Calculation.

The difference between the cubic centimeters of ammonium sulphocyanide solution expressed in decinormal strength required for the blank experiment and that required in the main experiment represents the A Value. In terms of N/10 silver-nitrate solution, the result is expressed as follows :

If a = C.C. N/10 silver nitrate solution used in precipitating the insoluble silver salts in the main experiment of A Value,

and b = C.C. of N/10 silver-nitrate solution used in the blank experiment,

then A Value = (a - b)

Estimation of B-Value.

200 C. C. of the filtrate "F" are transferred to an Earlenmer flask of 300 C. C. capacity and are exactly neutralised with semi-normal sulphuric acid solution using a 1% solution of phenolphthalein as indicator. The filtrate is then transferred to a 250 C. C. measuring flask and brought to the desired volume of 250 C. C.

To this are added 2 gms. of chemically pure solid silver sulphate. Due to the excess of silver sulphate, the precipitation takes place always in a saturated solution of silver sulphate which fact retards the possibility of any silver salts of fatty acids from caproic acid onward from going into solution while the silver butyrate remains in solution. It is shaken properly and kept in a bath for about an hour at a temperature of about 20° - 30° C. This is then filtered and 200 C. C. of this filtrate are transferred to the distilling flask of the Reichert-Polenske apparatus along with the addition of a pinch or two of powdered pumice stone. 50 C. C. of dilute sulphuric acid solution (prepared by dissolving 26 C. C. of chemically pure concentrated sulphuric acid in a litre of distilled water) are then added to the above. The distillation of the above-mentioned mixture is carried out as usual and exactly 200 C. C. of the distillate are collected in the B value receiver. This is then quantitatively transferred to an Earlenmeyer flask and the whole is titrated against deci-normal caustic potash solution using a 1% solution of phenolphthalein as indicator.

A blank experiment is also carried out exactly in a similar manner.

Calculation.

The result of B value is calculated as follows :

If c = C.C. of N/10 potash used in the main experiment of B value,

and d = C. C. of N/10 potash used in the blank experiment.

Then the B value = $(c-d)$.

N. B.—The method of A and B value estimation as given above is taken from D. Holde's "Kohlenwasserstoffe und Fette", 6th edition, 1924, pp. 621-623, and the modifications suggested by the present authors have been put in italics.

CHAPTER XII

The Detection of Adulteration of Butterfat (Ghee).

(Reprinted from Current Science, February, 1936.)

The adulteration of butterfat (ghee) has been penalised by all the Provincial Governments of India and some of these have already taken very serious steps to punish the dealers in this important article of food, whenever the adulteration has been detected and proved in a law-court. Every province has got a special Chemical Analyst, whose business it is to examine and report on the samples of ghee (as also other food-stuffs) submitted to him for report. The act dealing with the prevention of food adulteration empowers the trying magistrates to decide the cases before them on the strength of the reports submitted by the special officers. In the interests of the vast public, it is but necessary to punish those who sell adulterated ghee (as also other adulterated food-stuffs). The responsibility which rests on the Chemical Analysts to the various Governments is, therefore, very great indeed. In the interests of justice and also in the interest of the public for whom justice is administered, it is of paramount importance that the investigation of the adulteration must be both *scientific* and *correct*.

In our investigation of this problem, we have come across certain points which need a very careful consideration. The main problem is, what are the correct physical and chemical constants of butter and butterfat from the scientific point of view? What are the limits of these? How is the purity or impurity of both butter and

butterfat to be ascertained? Is there, in the first place, a correct knowledge of the composition of Indian butter or butterfat, from cows and buffaloes, either separately or mixed? Are the different Provincial Analysts in India agreed on a unanimous standard of the limits of constants of pure butter and butterfat? Has sufficient work been done on the subject in India and have the chemists concerned met and discussed their experimental data obtained from Indian samples? All these questions must be answered and decided before any sample is pronounced as adulterated.

The results obtained in this Laboratory have already been published in the form of a booklet entitled *Butterfat* (by N. N. Godbole and Sadgopal) wherein certain new methods have been suggested. It is, of course, necessary that the methods proposed by us should be carefully examined by the Provincial Chemists before they are made generally applicable. It is, therefore, important to examine the various standards adopted by the Government Chemists in the different provinces of India. We are thankful to the various Chemists who supplied to us the information which has been put together in the following Table.

Table No. 35.

A brief summary of Standards adopted in various provinces of India for the purity of "Butterfat" (upto the end of 1937.)

N. B.—(New standards have been introduced since. Authors).

Standards for mixed Butterfat.

No.	Name of the Laboratory	Refractive Index at 40° C. Butyro-reading	Reichert-Meissl Value	Remarks
1	Bengal, Government of	Not less than 40 and not more than 42.5	Not less than 28	Determination of Saponification value if necessary

(contd.)

Table No. 35—(contd.)

2	Bihar and Orissa, Govt. of	40 to 42	Not less than 23	Phyto terol acetate test to be negative in all cases
3	Bombay Corporation	40 to 44.5	Not less than 24	..
4	Calcutta Corporation	Not less than 40 and not more than 42.5	Not less than 28	Saponification value to be determined if necessary
5	Karachi Municipality	Not less than 40.5 and not more than 44.2	Not less than 24	Polenske value, Kirschner value and qualitative tests for hydrogenated oils are made when necessary
6	Lahore Municipality	Not less than 40 and not more than 41.6	24 to 32	Not more than 2.6 per cent. of free fatty acid allowed
7&8	Madras, Corporation of Madras, Government of	A general examination of the sample is made	Not less than 22, also should be above 27	Not more than 1 per cent. moisture: Sterol-acetate, Iso-oleic acid etc. Isolation of Sterol acetate, estimation or Iso-oleic acid and other tests for a thorough examination
9	Mysore, Government of	—	—	No Standards are fixed as yet
10	Nagpur, Municipality	From 40 to 46	From 19 to 36	—
11	New Delhi Municipality	—	—	No Standards are fixed as yet
12	Punjab, Government of	40 to 42	24 to 32	Baryta Value (Lallement's process to be negative. Free fatty acids to be not more than 2.8 per cent.)
13	Pusa, Agricultural Institute	Abbe's Scale 1.4524 to 1.4538	26 to 42	Saponification value and Iodine value (Hubl) are also determined if necessary
14	United Provinces— (a) Agra (b) Lucknow	Not less than 40 and not more than 51 at 25°C.	Not less than 28	Moisture to be not more than 1 per cent. and Saponification value to be determined if necessary

From the following table, it is clear that the different provinces in India are not only not unanimous in their criteria of the purity of butterfat, but they differ widely even in the limits of the values they have laid down. A student of science or a specialist in oils and fats will find the differences in the standards of different provinces too wide to be justified. Indeed, looking to the values tabulated above, it is clear that a sample which will be pronounced as pure by one Provincial Analyst will be dismissed as positively adulterated by another Provincial Chemist. It is high-time, therefore, that a conference of all the chemists interested in the investigation of butter and butterfat be called as early as possible to discuss :

(1) the limits of the physical and chemical constants of pure butterfat, and (2) to standardise the methods for the detection of the adulteration, both qualitative and quantitative.

Coming to the scientific aspect of the standards, just at present, the Reichert Meissl value and the refractive index (with the help of the Butyro-Refractometer) are the two main tests by which the purity of butterfat is ascertained in all the provinces. It is true that values like the Saponification Value, Iodine Value, Lallement's Baryta Value, Kirschner Value or the tests for iso-oleic acid and phytosterol acetate are used in certain laboratories as supplementary tests to confirm certain doubtful results and to enable one to draw a positive or a negative inference. In our opinion, the Reichert-Meissl Value which has a range for pure butterfat from 19 to 35 is too good to be used; instead of that, we have proposed that the so-called A and B values, (Bertram, Bos and Verhagen) which possess a very narrow range, should be used. These values have been found by us to be extremely satisfactory

in their results. From an analysis of nearly two hundred samples of cow and buffalo butterfats from all provinces of India, we have ascertained that the B value, which has a very small range, gives very reliable results. We would very much like that this be further examined by chemists to the Provincial Governments in India, with samples available in the different provinces.

The great difficulty in the analysis of butterfat has been that the various constants of pure butterfat possess a very wide range depending upon the nature of the animal, the season and the *type of food* that is given to it. It has been our experience that the A and B values and especially the B values offer the least range in the limiting values. It can be mathematically shown that whereas even a 5% adulteration of butterfat appreciably affects the B value, the adulteration of even 20%, under similar circumstances, cannot enable the chemists with the help of Reichert-Meissl value, etc., to draw any positive inference in pronouncing a sample as adulterated. We are not aware of any other laboratory in India where much preliminary work has been done on the application of A and B values for detecting the adulteration of butterfat quantitatively. Messrs. Carl Zeiss of Jena, in their latest German pamphlet pertaining to the use of Butyro-Refractometer, have been good enough to mention the work done by us at this University as a reference book on the subject.

The Reichert-Meissl Value, as adopted in India, is of doubtful value for another reason also. Most of the vegetable and animal oils and fats (excepting cocoanut, palm- kernel and butterfat) have a Reichert-Meissl Value which is almost negligible. But Dolphin oil, a kind of fish oil, has got a high Reichert-Meissl Value of 39 to 112 with the result that if this is hydrogenated and added

to butterfat (which we understand is being done), it will make the application of Reichert-Meissl value of very little importance in pronouncing a verdict on the question of adulteration.

The application of refractive index, as observed in the Butyro-Refractometer of Messrs. Carl Zeiss of Jena, is from our point of view of very great importance, not merely because of the readings it gives but because of the characteristic colour-fringes which have been observed by us (in spite of the compensating prism) as also by a few of the earliest workers and which have been discussed in detail in another chapter. We have drawn the attention of the numerous workers in this line to these characteristic colour-fringes and so far we have received no complaints to the contrary. Experiments are in progress in this University to photograph these coloured lines to show whether the sample of butterfat under examination is adulterated or not. The range of degrees in the Butyro-Refractometer as given by the different Provincial Governments is not in agreement with the observation which we have made and collected in our trials of a few hundreds of samples of pure butterfat. The range which we have observed for pure butterfat at 40°C. is from 40°—43.5° on the scale of the Butyro-Refractometer.

In some of the Provincial Laboratories (*Vide* U. P. Government standards) the observations are taken at 25°C. We fail to understand how a reading could be taken at 25°C. or why it should be taken at all at 25°C. when we know that many samples of pure butterfat have a melting point very much above 25°C. As is well known, no reading could be correctly taken in the Butyro-Refractometer unless the sample is *in a melted condition*, during

the process of examination. We have found in the case of many adulterated samples that the range of melting point exceeds 44.5°C . and then the characteristic colour-fringes—bluish green or orange red, etc.—betray the adulteration of the sample. For a qualitative test, which does not take more than a few minutes, we are of opinion that the observation of the Refractive Index *along with the Coloured Lines* is of great help in pronouncing an opinion on the purity of a sample.

Regarding the other values like the saponification value, iodine value, Kirschner value, sterols, etc., although these are valuable in themselves, we do not think that *directly* they are of much help. At best, they will render only *supplementary help*. But we would emphasise that the A and B values, if carried out carefully, will enable a worker to draw perhaps the most accurate inference. The other values because of their wide range cannot be of much help unless they are all *put together*.

It is imperative in the interests of national health that a very effective legislation should be enacted to stop the adulteration of butterfat, one of the most important food-stuffs of the vegetarian dietary. But at the same time it is equally desirable in the interests of science and justice that the standards adopted in various provinces should be thoroughly examined, corrected, and re-arranged in order to protect the legitimate interests of the dealers in this article.

N. B.—These old standards have been replaced by new ones which are discussed in the next chapter.

CHAPTER XIII

A criticism of the recent standards (given in Table No. 36) laid down by the Ghee Conference of the Government of India during 1937-38.

During the last two years, the Government have revised their old standards given in Chapter XII and new ones have been introduced. These are given in the following table.

Table No. 36.

Government standards for Butterfat fixed in 1937-38.

SCHEDULE III.—Normal physical and chemical constants of "Ghee" to which grade designation marks may be applied.

.....	Cow-Ghee Yellow label	Buffalo-ghee Blue label.	Special, Red label.	General, Green label.
Butyro-reading at 40 °C	40.5-42.5	40.5 to 42.5	40.5-42.5	40.5-43.5
Moisture % ..	Not more than 0.5	Not more than 0.5.	Not more than 0.5.	Not more than 0.75.
Sap value ...	222-226	226-234	222-234	220-236
Reichert-Meissl value.	26-28	Not less than 30.	Not less than 28.	Not less than 24.
Polenske value	1.5-2.5	1.0-1.75	1.0-2.0	0.5-2.5
Kirschner value	20-25	Not less than 25.
% Free fatty acid as Oleic acid.	Not more than 1.5.	Not more than 1.5.	Not more than 1.5.	Not more than 2.5.

An attempt has been made by the Ghee Conference of Government of India (vide Table No. 36) to differentiate between cow and buffalo ghee. The limits of the values for saponification, Reichert Meissl, Reichert Polenske and Kirschner values etc., as laid down by the Government of India are both unscientific and arbitrary. If the limits laid down in the Government's specifications are to be taken as final, then a very large number of *genuine* samples of cow and buffalo ghee will have to be thrown out as impure and adulterated. In our opinion, these limits as laid down are unjustifiable. Not merely that but in many cases a genuine sample of cow ghee because of certain values it possesses will have to be labelled as buffalo ghee and *vice versa*.

Regarding the standards laid down for the so called special and general brands of ghee, the limiting values are absolutely untenable and arbitrary. There is a large amount of literature available all the world over giving the minimum and maximum values of genuine samples of the butter fat of the cow and buffalo either pure or mixed. If these are compared with the standards laid down by the Indian Government, the only criticism that can be offered is that these standards are simply untenable.

Coming now to the detailed working of the laboratory experimentation of these values, the procedure proposed in the official bulletin¹ leaves much to be desired. It is difficult to understand why the Butyro-refractometer reading is confined to only 40.5-42.5 in the case of cow and buffalo ghees. Absolutely pure samples do give a reading *more than 42.5*. One does not understand on what grounds such samples can be excluded.

(1) Standard methods of analysis for testing and grading ghee 1938, published by the Government of India.

The estimation of moisture is done by heating the sample to constant weight at 105° C. This method is open to two objections. Firstly, the last traces of moisture are difficult to be expelled by this method due to the emulsified nature of the fat. Secondly, there is also the fear of volatilisation of the partially volatile butyric acid, aldehydes and ketones which may be present as products of rancidity. The loss due to these will be interpreted as being loss *due to moisture*. It is, therefore, recommended that this method of estimating moisture should be replaced by the modern method of using a high boiling solvent like Xylol as is done for oils, fats and soaps.

The limits of saponification value also are not altogether scientific but the difference can be ignored as the error is not very serious.

Regarding the Reichert Meissl and Reichert Polenske values, the limits as given in the Government specifications seem to be hardly tenable. It is difficult to understand why in the case of cow ghee, a range of 26-28 is accepted and in the case of buffalo ghee a range of "not less than 30" has been set up. This limit of the value is too good to be criticised. We wish here to refer to chapter No. VI in which we have fully discussed the comparative merits of Reichert Meissl, Reichert Polenske and Kirschner values. We would advocate without any hesitation the introduction of A and B values or Grossfeld's Butyric Acid number as a substitute for the Reichert Meissl and Polenske values for more than one reason. It is sometimes argued that the A and B values are a little elaborate and need expert handling in their laboratory manipulation. To our mind, this is absolutely no excuse. Arbitrary standards are no substitute for scientific accuracy. It should be remembered that the traders and commission agents handling

genuine ghee are being hit hard by the un-scientific and arbitrary standards of the Government.

The percentage of free fatty acids in the case of butter fat has been calculated as oleic acid. This method of estimating fatty acids is an obsolete one. As is well known, it is both scientific and logical to estimate the free acid in the case of butter fat as Butyric acid just as in the case of coconut oil, the free fatty acids are estimated in terms of lauric acid. In the case of oils and fats like ground-nut oil, sesame oil or lard etc., the estimation of free fatty acids as oleic acid is reasonable. But that is not the case with butter fat. Further if the percentage of free fatty acids estimated as oleic acid as allowed in the case of ghee by the Government standards is converted into the corresponding amount of Butyric acid, the butyric acid thus obtained comes to 0.5-0.75 per cent. in the case of the special and general brands of ghee. It is questionable whether such a percentage of butyric acid is permissible in its free form from a physiological point of view.

In view of the above mentioned remarks it is imperative that the Government standards should be over-hauled and brought in line with upto-date and scientific methods supported by the latest researches in the field of oil chemistry.

CHAPTER XIV

Rancidity of Butterfat, Its causes, nature, detection & Prevention.

Introductory.

The subject of rancidity *i.e.*, the development of an acrid taste and unpleasant aroma in oils and fats, rendering the same unfit for edible purposes, has attracted the attention of chemists for a very long time. It has been studied particularly with special attention to the decomposition of butter-fat on keeping.¹ Numerous explanations have been put forward from time to time leaving the subject still vague. The present authors have taken up the investigation of the problem systematically with special reference to butterfat only.

An investigation into the causes and nature of Rancidity.

General procedure and preparation of butterfat for examination.

A quantity of buffalo-butter, freed from milk-sugar and casein and made out of pure sterilised milk, was melted over a water bath and transferred to a separating

(1) Schmidt, Zeits. f. Hygiene u. Infektionskrankheiten, 1898, 183; Hanus, Zeits. f. Unters. d. Nahrge-u. Genussm., 324; Hanus and Stoky, Ibid, 806; Lydia Rabinowitsch, Jahrbuch d. Chem., 1899, 9, 237; Crampton, J. A. C. S., 1902, 711; Orla Jensen, Jahrbuch d. Chem., 1902, 12, 363; O. Laxa, Arch. f. Hygiene, 1902, 119; A. Nestrelajew, Zeits. f. Unters. d. Nahrge-u. Genussm., 1911, 431; M. Siegfeld, Milchw. Zentralbl., 1908, 530; Fernci, Zeits. f. angew. Chem., 1911, 2241; Shacht. Analyst. 1911, 597; Burr, Wolff, and Berberich, Zeits. f. Unters. d. Nahrge-u. Genussm., p. 200.

funnel. It was freed from its water-content and shaken thoroughly with freshly prepared anhydrous sodium sulphate. The moisture-free fat thus obtained was finally filtered through a hot-filter-funnel and then preserved in carefully cleaned and sterilised glass-stoppered bottles. For all further examinations this sample was employed.

The various physico-chemical properties as given in the following pages were all studied according to the methods given in the *Einheitliche Untersuchungsmethoden fuer Fett-und Wachsindustrie* (1930) except in the case of the determination of A and B-values for which the method as modified by the present authors was employed.

SECTION A. (Preliminary.)

TABLE No. 37.

*Physico chemical examination of the freshly prepared sample
of butter-fat.*

AS BUTTER-FAT :

Physical state.	Fully developed an characteristic butter aroma and taste.
Specific gravity at 15° C	0.9358
Solidifying point° C	20.6
Saponification value	234.0
Iodine value (Hanus)	26.54
Reichert Polenske value	0.7
Reichert Meissl value	28.0
A-Value	6.3
B-Value	33.0
Butyro-refractometric number at 40°C	42.6
Godbole-Sadgopal-Lines (Butyro-refractometric)	Colourless
Acid value	0
Hehner value	86.1
AS MIXED FATTY ACIDS :	
Specific gravity at 35°C	0.9050
Solidifying point C	33.5
Mean molecular weight of the fatty acids	252.1
Hehner value	86.1
Iodine value (Hanus)	36.1
ⁿ D at 60° C	1.4370
Glycerine (By acetic method)	8.8% (?)
,, (Calculated from Ester value)	12.8% (?)

Table No. 38.

Composition of the mixed fatty acids (As determined by the special combination method adopted by the authors.)

<i>Fatty acid.</i>		<i>Percentage</i>	
1. Butyric	4.0
2. Caproic	2.05
3. Caprylic	0.95
4. Capric	2.0
5. Lauric	4.0
6. Myristic	9.5
7. Palmitic	31.5
8. Stearic	12.0
9. Unknown	0.3
10. Oleic	29.5
11. Linoleic	4.4

Table No. 39 :—The fat was hermetically sealed in a thoroughly sterilised colourless glass tube freed from all air and moisture and kept exposed to diffused light for 93 days.

Table No. 40 :—The fat was kept in a sterilised porcelain dish placed in a vacuum dessicator containing water. The latter was evacuated thoroughly, covered with black paper, wrapped in a black piece of cloth and was kept in a dark almirah for 92 days.

Table No. 41 :—The fat was kept in a sterilised porcelain dish placed in a dessicator containing fused calcium chloride, covered with a black paper and wrapped in a black piece of cloth for 93 days.

Properties.	As per Table No. 37	Table No. 39 Light alone present.	Table No. 40 Moisture alone present.	Table No. 11 air alone present.
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AS BUTTER-FAT :

Physical state.		Very slight bitter taste & a slight nupleasan t aroma.	Aroma un- affected acid taste,	A slight acid taste. Un- pleasant aroma
Specific Gravity at 15°C	0.9358	0.9362	0.9362	0.9385
Solidifying point°C ..	20.6°C	19.95	19.85	20.45
Saponification value ..	234.0	235.0	238.2	234.1
Iodine value ...	26.54	26.85	24.62	20.95
Reichert Polenske value.	0.7	0.65	0.6	0.62
Reichert Meissl value	28.0	28.1	29.0	28.1
A-value ...	6.3	6.1	5.9	5.9
B value ..	33.0	33.6	35.7	35.7
Butyro-refractometric Number at 40°C.	42.0	42.2	42.15	42.15
Godbole-Sadgopal line. (Butyr-R-refracto).	colourless.	Very light violet.	very light violet.	Very light violet.
Acid value ..	0		8.9	0
Hehner value ..	86.1	85.75	84.75	86.5

AS MIXED FATTY ACIDS :

Specific Gravity at 35°C	0.9050	0.9072	0.9070	0.9100
Solidifying point°C ..	33.5°C	32.45	31.5	33.3
Mean molecular weight	252.1	251.15	242.8	250.7
Hehner value ..	86.1	86.0	85.7	86.0
Iodine value ..	36.1	37.25	29.0	27.2
n_D at 60°C	1.4370	1.4375	1.4390	1.4400
Glycerine (By acetin method)	8.6%	8.5%	6.5%	8.2%
Glycerine (calculated from Ester-value).	12.8%	12.8%	12.5%	12.8%

Conclusions.

1. Table No. 39 :—Light alone does not affect the fat. There is a slight change of taste. This taste is due probably not to the fatty matter but to the aromatic compounds like diacetyl etc. A change in the taste does not necessarily affect any of the physical or chemical characters.

2. Table No. 40 :—Moisture alone produces an acrid taste, lowers Iodine value, R. P. value, A value, percentage of glycerine, Hehner value and mean molecular weight of mixed fatty acids. It increases the acid value, saponification value, R. M. value and B value along with refractive index.

3. Table No. 41 :—Air alone lowers the Iodine value and increases the specific-gravity, R. M. value and B value. Taste is rendered acrid and an unpleasant aroma is developed. Glycerine content remains unaffected.

Table No. 42 :—The fat was kept in a sterilised porcelain dish placed in a vacuum dessicator containing water, evacuated and finally kept open to diffused light for 92 days.

Table No. 43 :—A similar experiment was continued for 115 days.

Table No. 44 :—Another experiment was continued for 167 days under similar conditions.

Properties.	As per table No. 37.	Moisture and Light present for		
		92 days	115 days.	167 days.
AS BUTTERFAT: Physical state.	Fully developed and characteristic butter arona and taste.	Table No. 42. Aroma practically unaffected. Taste acrid.	Table No. 43. Aroma unaffected. Taste very acrid.	Table No. 44. Aroma unaffected. Very strong acrid taste.

(contd.)

Table Nos. 42, 43 and 44.—(contd.)

Specific gravity at 15°C.	0.9358	0.9400	0.9400	0.9400
Solidifying Point°C...	20.6	19.8	19.6	19.3
Saponification value	234.0	236.0	236.95	237.6
Iodine value ..	26.54	25.9	25.4	25.0
Reichert Polenske value.	0.7	0.61	0.58	0.54
Reichert Meissl value.	28.0	28.95	29.1	29.95
A-value	6.3	6.1	6.0	5.74
B-value	33.0	33.5	34.2	35.0
Butyro-refractometric number at 40°C.	42.1	43.2	43.35	43.8
Godbole-Sadgopal-Line (Butyro-refracto)	colourless.	Light violet.	Light violet	Light violet
Acid value ..	0	9.3	9.85	11.65
Hehner value ..	86.1	82.0	81.5	80.3
AS MIXED FATTY ACIDS :				
Specific gravity at 35°C.	0.9050	0.9075	0.9075	0.9075
Solidifying point°C.	33.5	31.0	30.95	30.65
Mean molecular weight.	252.1	240.5	238.0	235.2
Hehner value,	86.1	84.2	83.75	83.0
Iodine value.	26.1	27.6	27.0	25.8
ⁿ D at 60°C.	1.4370	1.4380	1.4386	1.4392
Glycerine (By acetic method)	8.6%	6.35%	5.95%	5.6%
Glycerine (Calculated from Ester value).	12.8%	12.45%	12.4%	12.3%

Conclusion.

Tables Nos. 42, 43, and 44 :—Moisture and light together lower the solidifying point, Iodine value, R. P. value, A value and Hehner value along with the mean molecular weight of the fatty acids. They increase the acid value, saponification value, R. M. value and B value along with the refractive index. Taste gets acrid on keeping and glycerine content is lowered. Aroma is not affected very much. On keeping the sample for longer periods, the above-mentioned effects are exhibited strongly.

Table No. 45 —The fat was kept in a sterilised porcelain dish placed in a dessicator containing fused calcium chloride and finally exposed to diffused sunlight of the room for 92 days.

Table No. 46 :—The fat was kept in a dessicator containing water, covered with black paper, wrapped in a black piece of cloth and kept in a dark almirah for 90 days.

Table No 47 :—The fat was kept in a dessicator containing water and exposed to diffused light of the room for 90 days.

Properties.	As per table No. 47.	Table No. 45. Air & light present.	Table No. 46 Air and moisture present.	Table No. 47 Air, moisture and light present.
AS BUTTERFAT:				
Physical state.	Fully developed and characteristic butter aroma and taste.	Aroma affected taste very acid.	Strongly acid taste Very unpleasant tallowy smell.	Very pronounced acid taste and tallowy aroma
Sepecific gravity at 15°C.	0.9358	0.9488	0.9392	0.9358
Solidifying point°C.	20.6	20.4	19.2	19.0
Saponification value.	234.0	235.5	240.5	239.85
Iodine value.	26.54	19.95	19.75	19.15
Reichert-Pole n s k e value.	0.7	0.6	0.55	0.5
Richert-Meissl value.	28.0	28.0	28.0	30.2
A-value.	6.3	5.75	5.65	5.2
B-value.	33.0	36.35	36.8	37.5
Butyro-refractometric number at 40°C.	42.0	42.35	43.8	44.5
Godbole-Sadgopal-Line. (Butyro-Refracto)	colourless.	}	Light Violet.	}
Acid value	0		0.035	
Hehner value.	86.1	85.0	82.5	81.7

(contd.)

Table Nos. 45, 46 and 47. —(contd.)

AS MIXED FATTY ACIDS :				
Specific gravity at 35°C.	0.9050	0.9100	0.9105	0.9110
Solidifying point°C ...	33.5	33.0	30.1	30.2
Mean molecular weight.	252.1	248.0	235.5	230.35
Hehner value ..	86.1	85.5	83.2	82.5
Iodine value ..	36.1	25.5	23.4	22.0
ⁿ D at 60°C.	1.4370	1.4385	1.4410	1.4400
Glycerine (By acetin-method).
Glycerine (Calculated from Ester value.)	8.6%	8.2%	6.0%	5.6%
	12.8	12.8%	12.4%	12.4%

Conclusions.

1. Table No. 45 :—Air and light together lower the Iodine value, R. P. value, A value and Hehner value along with mean molecular weight of the mixed fatty acids. They increase the specific gravity, saponification value, Reichert-Meissl value and refractive index. Glycerine content remains practically unchanged. Presence of light has positively helped the action of air.
2. Table No. 46 :—Air and moisture together increase the Specific gravity, Saponification value, R. M. value, B value, Acid value and refractive index while the solidifying point as well as Iodine value, R. P. value and Hehner value are lowered along with the mean molecular weight of the mixed fatty acids.
3. Table No. 47 :—Light acts as a positive catalyst to accelerate the effect of air and moisture together on butterfat. For the effect of moisture and light only and no air, see table Nos. 42, 43 and 44 separately attached.

Table No 48 :—The fat was hermetically sealed in a big test tube and exposed to direct sunlight for 19 days.

Table No. 49 :—The fat was hermetically sealed in a big test tube and exposed to Mercury-vapour lamp-light for 102 hours.

Properties.	As per Table No. 37.	Table No. 48.	Table No. 49.
AS BUTTER FAT :			
Physical state.	Fully developed and characteristic butter aroma and taste	Direct sunlight. Acrid taste and tallowy aroma.	Mercury vapourlight. A very pronounced acrid taste and rancid aroma.
Specific Gravity at 15°C.	0.9358	0.9462	0.9382
Solidifying point°C. ..	20.6	19.19	19.0
Saponification value. ..	234.0	236.25	236.2
Iodine value ..	26.54	22.2	22.8
Reichert-Polenske value.	0.7	0.6	0.58
Reichert-Meissl value ...	28.0	30.85	29.85
A-value ...	6.3	6.0	6.0
B-value ..	33.0	35.82	35.0
Butyro - refractometric number at 40°C. ..	42.0	42.8	43.6
Godbole-Sadgopal - Line (Butyro-refracto) ..	Colourless.	light violet.	Violet.
Acid value ..	0	5.2	9.2
Hehner value. ..	86.1	84.5	83.8
AS MIXED FATTY ACIDS :			
Specific gravity at 35°C.	0.9050	0.9102	0.9120
Solidifying point°C. ..	33.5	32.35	32.55
Mean molecular weight ...	252.1	245.25	242.2
Hehner value. ..	86.1	84.8	83.95
Iodine value. ..	36.1	28.6	26.1
ⁿ D at 60°C. ..	1.4370	1.4392	1.4400
Glycerine (By acetin method)	8.6%	7.5%	7.15%
Glycerine (Calculated from Ester value).	12.8%	12.6%	12.1%

Conclusions.

1. Table No. 48 :—In direct sunlight, the fat gains in specific gravity, saponification value, acid value, R. M. value, B value and refractive index with a distinct change

in the colour fringes of Godbole-Sadgopal-Lines. The solidifying point, iodine value, R. P. value, A value, Hehner value, glycerine content and the mean molecular weight of the mixed fatty acids are lowered. Taste and aroma are affected very much.

2. Table No. 49 :—All the changes mentioned above have been affected in the same direction but comparatively within a very short time.

Table No. 50 :—A sample of the fat was treated with 0.5% of iron (Merck's extra pure metallic powder) and kept in a porcelain dish placed in a dessicator containing fused calcium chloride, evacuated, covered with black paper, wrapped in a black piece of cloth and kept in a dark almirah for 23 days.

Table No. 51 :—Another sample was kept for the same number of days and in contact with 0.5% of aluminium (Merck's extra pure metallic powder).

Table No. 52 :—Another sample was kept under similar conditions and for the same number of days but in contact with 0.5% of tin (Merck's extra pure metallic powder).

Table No. 53 :—Another sample was kept under similar conditions and for the same number of days but in contact with 0.5% of copper (Merck's extra pure metallic powder).

Properties.	As per Table No 37.	At a Temperature of 25—30°C In absence of air, moisture and light but in presence of 0.5% of			
		Iron. Table No. 50.	Aluminium Table No. 51.	Tin. Table No. 52.	Copper Table No. 53.
AS BUTTER-FAT :					
Physical state ..	Fully developed and characteristic butter aroma and taste.	Taste and aroma unaffected.	Aroma affected slightly.	Slight change in aroma and taste.	Tallowy aroma & metallic saltish taste.
Specific gravity at 15°C.	0.9353	0.9360	0.9359	0.9362	0.9388
Solidifying Point°C	20.6	20.6	20.62	20.45	19.0
Saponification value	234.0	234.1	234.5	234.2	235.0
Iodine value ...	26.54	26.2	26.15	26.75	22.0
Reichert- Polenske value.	0.7	0.68	0.68	0.72	0.6
Reichert-Meisner value.	28.0	28.2	28.58	28.3	30.0
A-value ..	6.3	6.3	6.2	6.35	5.0
B-value ...	33.0	33.0	33.2	33.0	34.5
Butyro-rafractometric number at 40°C.	42.0	42.0	42.15	41.8	43.2
Godbole-Sadgopal—Line. (Butyro-refracto.)	Colourless	Very light violet.		Light Violet.	
Acid value ...	0	0	0	0	2.85
Hehner value ..	86.1	86.0	85.5	85.7	82.0
AS MIXED FATTY ACIDS :					
Specific gravity at 35°C	0.9050	0.9072	0.9070	0.9060	0.9088
Solidifying point°C	33.5	33.8	33.2	33.2	31.0
Mean molecular weight.	252.1	252.35	250.0	251.5	246.0
Hehner value ..	86.1	86.2	86.0	86.45	85.2
Iodine value ..	36.1	35.8	36.24	34.78	32.28
n _D at 60°C ...	1.4370	1.4365	1.4370	1.4375	1.4400
Glycerine (By acetic method).	8.6%	8.35%	8.25%	8.2%	7.0%
Glycerine Calculated from Ester-value)	12.8%	12.8%	12.5	12.8%	12.5%

Conclusions :

1. Table No. 50 :—In the absence of air, moisture and light, iron does not bring about any appreciable change.
2. Table No. 51 :—In the absence of air, moisture and light, aluminium does not bring about any appreciable change.
3. Table No. 52 :—In the absence of air, moisture and light, tin does not bring about any appreciable change.
4. Table No. 53 :—In the absence of air, moisture and light, copper changes taste and aroma fully along with an increase in specific gravity, R. M. value B value, saponification value, acid value and refractive index. A corresponding fall is observed in the solidifying point R.P. value, A value and Hehner value along with the mean molecular weight of the mixed fatty acids. Glycerine content is affected appreciably.

Table No. 54 :—The fat was kept for 23 days mixed with 0.5% of iron in a porcelain dish placed in a dessicator containing water and exposed to the average diffused light of the room.

Table No. 55 :—The same experiment was repeated with 0.5% of aluminium and under the same conditions.

Table No. 56 :—Another experiment under similar conditions but with 0.5% of tin was repeated in this case.

Table No. 57 :—Another experiment with 0·5% of copper under similar conditions was repeated in this case.

Properties.	As per Table No. 37.	At a Temperature of 25-30°C in presence of air, moisture and light and 0·5% of			
		Iron. Table No. 54.	Aluminium. Table No. 55.	Tin. Table No. 56.	Copper. Table No. 57.
AS BUTTERFAT:					
Physical state ...	Fully developed. and characteristic butter aroma and taste.	Aroma affected slightly.	Taste and aroma affected slightly.	Aroma and taste affected slightly.	Taste and aroma affected very much. Colour turned green.
Specific gravity at 15°C.	0·9358	0·9 60	0·9362	0·9358	0·9466
Solidifying point°C	20·6	19·95	19·8	20·2	19·0
Saponification value	234·0	234·1	233·8	235·0	238·0
Iodine value ...	25·54	26·25	26·35	26·2	24·2
Reichert-Polenske value.	0·7	0·69	0·7	0·72	0·61
Reichert - Meissl value.	28·0	28·15	28·0	28·5	42·95
A-value ...	6·3	6·2	6·0	6·2	5·9
B-value ...	33·0	33·5	33·75	33·0	36·95
Butyro-refractometric number at 40°C.	42·0	42·0	42·25	41·75	42·9
Godhale-Sadgopal — Line. (Butyro Refracto.)	Colourless	Very light violet.	Light	Violet	Violet
Acid value ...	0	0	0·25	0	9·35
Hehner value ...	86·1	85·5	85·6	85·25	83·65
AS MIXED FATTY ACIDS:					
Specific gravity at 35°C.	0·9050	0·9070	0·9072	0·9073	0·9120
Solidifying point°C	33·5	33·2	33·25	33·5	30·0
Mean molecular weight.	252·1	251·0	250·0	251·45	245·5
Hehner value ...	86·1	86·	86·0	86·2	85·1

(contd.)

Table Nos. 54—57.—(contd.)

Iodine value	..	36.1	34.2	34.0	34.3	32.1
n at 60°C	...	1.4370	1.4370	1.4365	1.4378	1.4388
D						
Glycerine (By acetic method)		8.6%	8.2%	7.8%	8.0%	6.5%
Glycerine (Calculated from Ester value).		12.8%	12.8%	12.8%	12.8%	12.4%

Conclusions :

1. Table No. 54 :—In presence of air, moisture and light, iron does not affect the fat appreciably.
2. Table No. 55 :—In presence of air, moisture and light, aluminium brings about a slight change.
3. Table No. 56 :—In presence of air, moisture and light, tin brings about a slight change.
4. Table No. 57 :—In presence of air, moisture and light, copper shows a clear increase in the specific gravity, saponification value, and refractive index while a marked decrease is observed in the case of iodine value, R. P. value, A value, Hehner value, mean molecular weight of the fatty acids, glycerine content and solidifying point.

Table No. 58 :—The fat was kept with 2% of casein (separated from milk) in a vacuum desiccator containing fused calcium chloride, evacuated thoroughly, covered with a black piece of cloth and kept in a black almirah for 92 days.

Table No. 59 :—The fat was mixed with 2% of casein (prepared from milk) and kept in a dessicator containing water and exposed to diffused roomlight at a temperature of 20°-25°C for 90 days.

Table No. 60 :—The above experiment was repeated at 25-35°C for 90 days and under similar conditions.

Properties.	As per Table No. 37.	Table No. 58 Table No. 59 Table No. 60 Casein Present in		
		Absence of air moisture and light.	Presence of air, moisture and light & at 20-25°C.	Presence of air, moisture & light & at 25-35°C.
AS BUTTERFAT :				
Physical state ..	Fully developed and characteristic butter aroma and taste.	Aroma unaffected. Taste curdy.	Strong rancid aroma. Bitter & acrid taste.	More pronounced rancid odour. Extremely bitter & acrid taste.
Specific gravity at 15°C.	0.9358	0.9360	0.9380	0.9385
Solidifying Point°C ..	20.6	20.5	19.0	19.0
Saponification value..	234.0	234.1	240.0	244.8
Iodine value ...	26.54	26.1	20.0	1.90
Reichert-Polenski value.	0.7	0.64	0.5	0.45
Reichert-Meissl value	28.0	28.0	30.0	32.2
A-value ...	6.3	6.25	5.0	4.65
B-value ...	33.0	33.4	36.5	38.2
Butyro-refractometric number at 40°C.	42.0	42.1	44.0	45.0
Godbole-Sa d g o p a l (Line.) Butyro-Refracto.	Colourless.	Very light violet	VIOLET	
Acid value ...	0	0	20.82	38.8
Hehner-value ..	86.1	85.65	80.0	78.8
AS MIXED FATTY ACIDS :				
Specific gravity at 35°C.	0.9050	0.9072	0.9108	0.9115
Solidifying point°C ...	33.5	33.35	30.25	29.25
Mean molecular weight	252.1	251.85	232.0	225.5
Hehner value ...	86.1	86.25	82.5	80.1
Iodine value ...	36.1	35.0	21.1	18.9
n _D at 60°C ..	1.4370	1.4372	1.4402	1.4402
Glycerine (By acetin method)	8.6%	8.35%	6.0%	5.5%
Glycerine(By calculation from Ester value).	12.8%	12.8%	11.9%	11.2%

Conclusions.

1. Table No. 53:—Casein in absence of air, moisture and light does not affect the fat in any way.
2. Table No. 59 :—Casein in presence of air, moisture and light increases very much the specific gravity, saponification value, Reichert-Meissl value, B value, Acid value and refractive index with a distinct change in the Godbole-Sadgopal-Lines. The solidifying point, iodine value, R. P. value A value and Hehner value are lowered along with the mean molecular weight of the mixed fatty acids. Glycerine content decreases. Taste and aroma are affected to a very great extent.
3. Table No. 60 :—At higher temperatures, further changes as mentioned above and to an increased extent are observed.

Table No. 61 :—The fresh butterfat was mixed with 1% of a very rancid sample of butterfat (possessing both the taste and the aroma rancidities) and kept in a vacuum dessicator containing fused calcium chloride, covered with black paper, evacuated thoroughly, wrapped in a black piece of cloth and kept in that state in a dark almira for 41 days.

Table No. 62 :—The fresh butterfat was mixed with 1% of a strongly rancid sample of butterfat as mentioned above and was kept in a dessicator containing water and placed in diffused light for 60 days.

Table No. 63 :—A sample of the fat was bottled in a thoroughly sterilised bottle and sealed. It was then pasteurised at 125°C in an autoclave for two hours. The fat thus prepared was kept in a dessicator containing water and placed exposed to diffused light in a room for 90 days.

Properties.	As per Table No 37	With 1 % rancid fat in		Air, moisture and light present in a sterilised sample.
		absence of air, moisture and light.	presence of air, moisture and light.	
		Table No. 61	Table No. 62.	Table No. 63.
AS BUTTER FAT:				
Physical state.	Fully developed characteristic Butter aroma and taste.	Taste slightly affected.	Very pronounced acrid taste and tallowy aroma.	Pronounced acrid taste and tallowy aroma.
Specific gravity at 15°C	0.9358	0.9355	0.9400	0.9395
Solidifying point°C ..	20.6	20.7	18.85	19.0
Saponification value	234.0	233.5	240.0	239.9
Iodine value ...	26.54	26.6	20.0	20.0
Reichert-Polenske value	0.7	0.7	0.45	0.51
Reichert-Meißl value	28.0	28.2	30.8	30.1
A-value ...	6.3	6.3	5.0	5.1
B-value ...	33.0	33.4	35.8	35.8
Butyro-refractometric number at 40°C	42.0	42.3	44.0	43.65
Godbole-Sadgopal-Line (Butyro-Refracto.)	.. colourless.	Very light violet.	VIOLET	
Acid value ..	0	1.22	15.8	15.34
Hehner value ...	86.1	86.32	80.0	81.7
AS MIXED FATTY ACIDS:				
Specific gravity at 35°C	0.9050	0.9070	0.9100	0.9108
Solidifying point°C ...	33.5	33.65	30.0	30.3
Mean molecular weight	252.1	252.0	239.0	232.5
Hehner value ..	86.1	86.4	82.5	82.9
Iodine value ...	36.1	36.5	22.0	22.5
ⁿ D at 60°C ..	1.4370	1.4374	1.4422	1.4415
Glycerine (By acetin method).	8.6%	8.2%	6.0%	6.25%
Glycerine (Calculated from Ester value).	12.8%	12.7	12.2%	12.3%

Conclusions.

1. Table No. 61 :—In absence of air, moisture and light, addition of rancid fat to the fresh one affects the taste and increases the free acidity.
2. Table No. 62 :—Addition of rancid fat to the fresh one acts as a positive catalyst hastening the effect brought about by air, moisture and light together on the fat.
3. Table No. 63 :—In spite of thorough pasteurisation and sterilisation, mere presence of air, moisture and light were sufficient to render the fat rancid.

Table No. 64 :—The butterfat was kept in a vacuum desiccator containing phosphorus-pentoxide, in a dark almirah for 307 days. The minimum and maximum temperatures during this period ranged between 20.05° — 30.0° C.

Table No. 65 :—The fat was kept in a sterilised porcelain dish placed in a dessicator containing calcium chloride and soda-lime, covered with black paper and was kept wrapped in a black piece of cloth for 90 days.

Table No. 66 :—The fat was salted with 2% of sodium chloride and kept in a dessicator containing water and exposed to average diffused light of the room for 60 days.

Properties.	As per Table No. 37.	Table No. 64.	Table No. 65	Table No. 66.
		absence of air, light and moisture.	presence of air deprived of carbon-dioxide.	2% common-salt in presence of air, moisture and light.
AS BUTTER FAT :				
Physical state	Fully developed and characteristic butte aroma and taste.	Taste slightly turned bitter.	Slight change in taste and aroma.	Aroma affected slightly but no effect on the taste.
Solidifying point°C ..	20.6	20.8	20.45	20.4
Specific gravity at 15°C	0.9358	0.9356	0.9380	0.9360
Saponification value..	234.0	234.0	234.4	234.0
Iodine value ..	26.54	26.55	20.75	27.0
Reichert-Polensko value	0.7	0.73	0.6	0.68
Reichert-Meißl value	28.0	28.24	28.25	28.0
A-value ..	6.3	6.3	5.95	6.3
B-value ..	33.0	33.0	35.3	33.0
Butyro-refractometric number at 40°C.	42.0	42.22	42.1	42.0
Godbole-Sadgopal--Line. (Butyro-Refract.)	Colourless	Colourless	Very light violet.	Colourless
Acid value ..	0	0	8.9	0
Hehner value ..	86.1	85.75	54.75	83.5
AS MIXED FATTY ACIDS :				
Specific gravity at 35°C	0.9050	0.9080	0.9095	0.9080
Solidifying point°C ..	33.5	33.6	33.35	33.35
Mean molecular weight	252.1	253.0	250.78	252.0
Hehner value ..	86.1	86.24	86.0	86.0
Iodine value ..	36.1	36.34	27.35	36.2
ⁿ D at 60°C ..	1.4370	1.4372	1.4412	1.4368
Glycerine (By acetin method).	8.6%	8.4%	8.42%	8.35%
Glycerine (Calculated from Ester V.)	12.8%	12.8%	12.8%	12.8%

Conclusions.

1. Table No. 64 :—In the absence of air, moisture and light the fat remains almost unaffected. There is a slight change of taste, this change being due probably not to the fatty matter itself but to the aromatic compounds like di-acetyl etc. This latter change need not affect any of the physical or chemical properties.

2. Table No. 65 :—Presence or absence of atmospheric carbon-di-oxide does not affect the course of reaction of ordinary air on the fat as given in Table No. 41.
3. Table No. 66 :—Salt acts as a preservative against the action of air, moisture and light together on the fat.

Table No. 67.

Separation of the mixed fatty acids of the rancid fat as obtained in experiment mentioned in Table No. 47.

The composition of the mixed fatty acids of the completely rancid fat as obtained in experiment No. 47 was found out by the same method as was adopted in the case of fresh butterfat mentioned in Table No. 38.

	Fatty acids.	Percentage in rancid fat.	Approximate percentage in fresh fat as given in Table No. 38.
1.	Butyric	6.5	4
2.	Caproic	3.0	2
3.	Caprylic	3.0	1
4.	Capric	3.75	2
5.	Lauric	3.8	4.0
6.	Myristic	9.2	9.5
7.	Palmitic	30.8	31.5
8.	Stearic	11.75	12.0
9.	Unknown	0	0.3
10.	Oleic	23.0	29.5
11.	Linoleic	3.0	4.4
	Glycerine (By acetin method) ..	5.6%	8.6%
	„ (Calculated from Ester value)	12.4%	12.8%

Conclusion.

Percentage of lower fatty acids from C₄ to C₁₀ has increased from 9% to 16.25% and more or less a corresponding decrease has been affected in the percentage of unsaturated acids (from 35.9% to 26.0%) especially in the case of oleic acid.

Table No. 68.

The following are the acid values of some freshly prepared oils and fats not showing any rancid behaviour as tested by aroma, taste and Kreis reaction.

1.	Mutton Tallow	0.46
2.	Lard	0.55
3.	Coconut oil	1.20
4.	Mahuwa oil	7.55
5.	Groundnut oil	3.2
6.	Castor oil	1.25
7.	Sesame oil	2.35
8.	Almond oil	1.43

Conclusion.

The terms "Acidity" and "Rancidity" are not co-terminous.

Table No. 69.

Determination of Acetyl values of rancid samples of butterfat.

Refer to the sample in Table number.	Nature.	Acid value.	Acetyl value.
37	Original fresh sample	0	0
53	0.5% Cu in absence of air, light and moisture.	2.85	2.3
48	Direct sunlight	5.2	5.5
40	Moisture alone present	8.9	7.8
49	Mercury-vapour light	9.2	8.1
42	Moisture and light present for 92 days	9.3	8.3
43	do. do. 115 days	9.85	8.5
44	do. do. 167 days	11.65	8.8
46	Air and moisture present	13.4	9.4
47	Air moisture and light present without sterilisation.	15.1	9.6
63	Air, moisture and light present with sterilisation.	15.34	9.8
62	1% of old rancid sample added in presence of air, moisture and light.	15.8	10.0
59	In presence of air, moisture and light besides casein at 20-25°C.	20.8	11.0
60	In presence of air, moisture, light and casein at 25-35°C.	38.8	20.3

Not examined before :-

Obtained as a nine year old sample from a Hindu Vaidya (Doctor in Ayurveda). 46.3 29.3

Conclusion.

Rancid samples of butterfat show acetyl-values which go on increasing with increasing rancidification. Pure butterfat has no acetyl-value.

Table No. 70

A study of the butter-fat giving an idea of the effect of "Aging" on its weight.

The fat kept for 92 days in presence of air, and light according to the Table No. 45 was weighed for this experiment after a definite interval. The observations noted are given below :—

Weight on the first day	100,0000 gms.
Weight after keeping for 10 days		100,2775 gms.
Do.	30 days	100·8327 gms.
Do.	40 days	101·0110 gms.
Do.	50 days	101·3885 gms.
Do.	55 days	101·5275 gms.
Do.	60 days	101·6658 gms.
Do.	65 days	101·8055 gms.
Do.	70 days	101·9442 gms.
Do.	75 days	102·0841 gms.
Do.	80 days	102·2220 gms.
Do.	85 days	102·3610 gms.
Do.	90 days	102·5002 gms.

Conclusion :—There is a gradual increase in the weight the fat proportionate with time.

Section B.

(Discussion.)

Before arriving at a general conclusion as to the causes responsible for the development of rancidity in butter fat, it is necessary to discuss the individual factors a little more in detail. Regarding the action of light, it is a well known fact that it destroys the minute traces of chromogenetic substances. As a matter of fact, most of the colour

reactions erroneously ascribed to light owe their origin to the presence of colouring matter in oils and fats. Wagner, Walker and Ostermann found oxygen unnecessary to produce rancidity. According to them, light alone is sufficient.¹ This view of the above workers is further supported by the work of Jorissen,² who found hydrogen peroxide in the case of certain oils and fats exposed to light. The presence of the peroxide is further supported by Deitz³ and A. Tschirch & A. Barben.⁴ The experiment as given in Table No. 39 conducted by the present authors does not support the above view. Light alone has been found to play no part in the production of rancidity as such. A study of Table Nos. 42, 43, 44, 45 and 47 goes to prove conclusively that the only part played by light in case of rancid fats and oils is that of a positive catalyst. Exposure to direct sun-light and Raman's Mercury-Vapour Lamp (Table Nos. 48 and 49) have given results which, of course, support the view of the previous workers. But in common cases of rancidity, these special lights are not allowed to function. Diffused sun-light as such, therefore, alone is not sufficient to produce rancidity. That is also the view of the Report of the Food Investigation Board for the year 1929, by Lea, wherein it is stated (p.30) that the action of light consists exclusively in accelerating oxidation of the fat.

The study of the action of air alone shows that the two values affected appreciably are the Sp. Gr. and the iodine Values. While the former has increased, the latter has been lowered considerably. The explanation is very simple. Air has acted, in absence of other factors, clearly

(1) Z. Nahr. U. Genusam., 25, 704, 1913.

(2) Chem. Ztg. 22, 162, 1898.

(3) Chem. Ztg., 29, 705, 1905.

(4) Chem. Umschau, 1924, 31, 141 ; J. 1924, 43, 719-B.

as an oxidising agent giving rise to oxy-acids which lower the iodine value and naturally thicken the fat giving a higher Sp. Gr. It could not affect the aroma and taste to any appreciable extent. That clearly proves the inefficiency of air alone in the producing any effects of rancidity. Lewkowitsch¹ has proved in the case of linseed oil kept for 13 years in bulk and kakao oil kept in a sealed tube for ten years that no rancidity had set in.

An analysis of air surrounding butter before and after cold storage has shown that the oxygen content decreased and the CO₂ content increased, indicating oxidation change in the fat. The quantity of CO₂ found was directly proportional to the amount of non-fatty material present in the butter and the conclusion was drawn that the unpleasant flavours of the rancid butter are produced by the oxidation, not of the fat itself, but of the non-fatty ingredients.² This view seems to be quite reasonable. But this factor is not responsible for the spoilage of pure butter fat free from practically all the non-fatty ingredients. Again the view taken up by some workers that the butter fat undergoes a sort of hydrolytic change due to the action of a weak organic acid—carbonic acid of the atmosphere³—also does not hold good in view of the results given in Table No. 65. Development of oxidation products with peroxide character (tested with potassium iodide or Titanium Sulphate) is only a very crude expression for spoilage. The presence of the peroxides could not be proved sometimes even in strongly rancid fats.

(1) J. S. C. I., 1899, 557.

(2) D. C. Dyer, J. Agri. Res. 1916, 6, 927.

(3) Green : *The Soluble Ferments and Fermentation*, Cambridge, 1899 ; Lewkowitsch and Meeleod, *Proc. Roy. Soc.* 1903, 72, 31 ; cp. Chapter 2 ; Lewkowitsch, "Les Corps Gras. Conference," *Bull. Soc. Chim. de France*, 1909, 15.

The case of the action of moisture alone is remarkably different. Besides affecting the physico-chemical constants to a very large extent, it imparts to the fat a clear acrid taste, which is always characteristic of rancid butter-fat. This action is enhanced all the more, just like that of air and light together, in the presence of light which acts as a positive catalyst. We have already seen (Table No. 64) that no trace of rancidity is noticeable in the case of fat kept for a long time in absence of air, light and moisture. All these facts put together go to point to a very simple explanation of the causes responsible for rancidity in butter fat. Rancidity, both of taste and odour, is very prominent in the case of the sample exposed to the combined action of air and moisture together, (Table No. 46). This action is further activated by the presence of light acting as a positive catalyst. (Table No. 47). Therefore the decomposition of the fat during rancidification seems to proceed in two stages :

1. Hydrolysis of the glycerides and consequent formation of free fatty acids and glycerol due to the action of moisture and

2. Oxidation of the acids and glycerol thus formed and inter-action of the oxidation products under the influence of air.

In all such cases light and bacteria appear to play the part of positive activating catalysts. The effect is all the more pronounced in the presence of non-fatty matter. Salt acts as retarder or preservative (Table No. 66). In this connection, it has already been noticed that the increase in acidity at 15°C is greater in the case of unsalted butter than the salted one as the latter loses its moisture due to salting.¹ That clearly establishes the "preservative"

(1) R. M. Washburn and A. C. Dahlberg. *J. Dairy Sci.*, 1917, 1, 114-26 ; *Bull. Agric. Intell.* 1918, 9, 998-99.

action of the salt. The process of rancidification is accelerated very much by the presence of free fatty acids and also traces of the previously rancid fat (Tables Nos. 61 and 62). In the case of other oils and fats, the colouring matter contained therein has been suspected to play some part in their decomposition. Butter fat, being practically colourless forms an exception to this contention. In this connection it is worth nothing what part, if any, is played by the phosphatide, non-fatty lecithin-like matter, during this decomposition.

Recent literature published on the problem of rancidity suggests the following explanation :—

“Oleic rancidity of fats is exclusively a chemical process which according to A. Tschirch and A. Barben¹ as well as according to W. C. Powick consists primarily of an autoxidation of the reactive double bonds of the unsaturated glycerides; peroxides are formed, concerning the structure and kind of which almost nothing is known. The record step which follows (influenced by light, moisture, positive and negative catalyst) depends upon the splitting off of the fugitive or “In-between” products. Fundamentally it has been pretty well-established that the predominating processes are the formation of low molecular substances having acidic, aldehydic and ketonic characters. The glyceryl part of the molecule does not appear to enter the reaction. The primary or oxidative and the secondary or the compound-forming phases appear to proceed simultaneously. Scala² as well as Powick give the most importance of responsibility for the rancid odour to heptaldehyde.³”

(1) Schwiliz. Apoth. Ztg. 60, 281, 1924.

(2) Staz. sperm. agrar. Ital. 30, 613, 1897.

(3) Kurt Taufel and Josef Muller; Zeitschrift, Untersuchung Lebensmittel, 60, 473.

Thus a very important question is raised as to the final nature of the decomposition products in a rancid butter fat. According to Scala¹, oleic acid is primarily set free in rancid oils and fats. He also isolated the decomposition products of rancid fats and by oxidation got acids like Oenanthylic, Pelargonic, Butyric, Caprylic and Capric. Another paper also suggests the presence of acetic acid. The one fact admitted on all hands is that free fatty acids are always present in case of rancid fats. But that does not mean that its converse is also equally true. Table No. 63 clearly shows that the terms "Acidity" and "Rancidity" are not coterminous. The explanation for this is to be found in the following extract:—

The presence of very small quantities of free fatty acids which are always found in even the freshest vegetable oils and fats is due to the action of enzymes present in oleiginous seeds on the glycerides stored in the seeds.²

Similarly, the presence and increase of free fatty acids has been proved to have no relation with the intensity of the Kreis reaction. In this connection, old references suggest that free fatty acids do not grow rancid. But most recent experiments do not support this view-point and clear rancidity has been marked in cases of fatty acids like oleic and linolic. Grogen³ has again very clearly shown that fatty acids on keeping in the open air have broken down to lower fatty acids. In support of this Salkowski⁴ found in a sample of oleic acid kept for years a crystalline acid melting at 48°C. This, by the way,

(1) Staz. Sperium, Agri. Ital. 1897, 30, 613.

(2) Liebermann, Borichte, 1904, 1579 ; Bio. Chem. Zeitsch. 1909, 305.

(3) J. S. C. I., 1889, 202 ; Gohn ; Chem. Zeit., 1907, 855.

(4) Fettehrift sum Virchow—Jubilaum, 1918, 19.

explains the increase in weight of the butter fat on aging (Table No. 70). Nestrelajew¹ also found that on exposing a butter fat to air and light for 107 days, iodine value decreased and saponification value increased along with total weight. A. Genthe has shown that with advancing oxidation, the splitting of the molecules of oleic acid makes greater progress though in the beginning a decrease in weight manifests itself. More and more volatile decomposition products are formed whose escape counteracts and increases in weight. According to Hepburn,² the available evidence would suggest that during a comparatively short period of time, there is a tendency for volatile acids to be produced as oils become rancid, but under certain conditions these may be consumed or decomposed by biological agencies. The some-what contradictory results which have been published are probably due to different types of organisms causing varying types of decomposition.

The authors have come to the following conclusions in view of all that has been stated above :—

(1) During rancidification, the glycerol content of the fat is lowered showing thereby that it positively does take part in the process.

In this connection, it should be noted that the Acetin method of determining glycerol has given very low results, still one thing is clear and that is that on rancidification, glycerol does decrease.

(2) The analysis (Table No. 67) of the mixed fatty acids of a rancid sample (Table No. 47) reveals an increase in the following :—

(1) *Milchwirtschaftl. Zentr.* 1910, 6, 1-8; *Chem. Zentr.* 1910, 1, 755,

(2) Seventh International Congress of Applied Chemistry, 1909, Sec. VIII-C p. 268.

	per cent. increase
a. Butyric Acid	2.5
b. Caproic Acid	0.95
c. Caprylic Acid	2.0
d. Capric Acid	1.75

(3) The same examination reveals a decrease as follows :—

Fatty Acid	Per cent. decrease
a. Lauric	0.2
b. Myristic	0.3
c. Palmitic	1.3
d. Stearic	0.25
e. Acid X. (Unknown)	0.3
f. Oleic	6.5
g. Linolic	1.4

(4) There is a loss of about 2% in the content of the mixed fatty acids.

Another very much-controvertial factor held responsible for causing rancidity is the presence and action of micro-organisms or the enzymes resulting from their development¹. Against this view, are very remarkable observations of Ritsert and a number of other workers leading to the conclusion that :—

(1) C. A. Crampon. J. A. C. S. 1902, 24. 8. 711-19; O. Jenson. Centr. Bl. f. Bakter. U. Parasitenk, 8, 2, 11-16, 42-46, 74-80, 107-114, 140-144, 171-174, 243-252, 278-281, 309-312, 342-346, 367-369, 406-409; Chem. Centr. 1902, 1, 6, 362; 9, 337-338; 10, 599; 11, 678; 13, 771; 15, 889; 18, 1071-1072. Jaxa Arch. f. Hyg., 1902, 41, 1191; W. B. Coules and C. H. Eckles J. Dairy Sci. 1917, 1, 347-55; Bull. Agri., 1918, 9, 992-93; Green: The Soluble ferments and fermentation; Lewkowitsch and Macleod, Proc. Roy. Soc. 1903, 72, 31; cp. Chapter 2; Lewkowitsch, "Les Corps gras. Conference." Bull. Soc. Chim. de France, 1909, 15; L. A. Rogers, U. S. Dept. Agri. Bureau of animal Ind. Bull. No. 57.

a. Pure lard could not be turned rancid by bacteria, aerobic, or anaerobic.¹

b. Micro-organisms excepting fat-splitting organisms play no part in the production of rancidity in the case of butter fat.

c. Butyric ferment does not make butter rancid.²

d. In the case of pure fats, micro-organisms quickly die for want of a suitable medium.³

The experiments conducted by the present authors proved it beyond any doubt that micro-organisms, enzymes or bacteria cannot be held responsible as initial starters of the process of rancidity. Rancidity ought to have been noticed in the case of butter fat kept according to tables Nos. 64 and 61., if any of these factors were responsible for rancidity. Again an examination of table No. 63 goes to show that in spite of a thorough pasteurisation and sterilization, characteristic rancidity could be noticed. All these facts put together establish the fact that the above mentioned factors are not responsible for causing rancidity. Of course, once the game has been started by moisture, then air, enzymes, micro-organism and bacteria, as good opportunists, may come in and side with rancidifying factors as positive catalysts. Warmth (a temp. of 20-35°C.) has been found to accelerate the growth of the organisms, once introduced.

Sterilized fats etc., free from micro-organisms may develop ketone rancidity (positive reaction according to Taufel and Thaler) on storage, the process being accelerated by light and heat. Fatty acids from C₅ upwards,

(1) Untersuchungen über d. Ranzigwerden der Fett, Inaug. Dissert., Berlin, 1890.

(2) W. Hagemann. Rep. An. Chem 3, 156.

(3) W. N. Stokoe J. S. C. I., 75T., April 30, 1921.

glycerol and soap—K-Laurate—give the Taufel ketone reaction¹ after irradiation volatile acidic products being formed in the case of the higher fatty acids and glycerol.

There is no doubt that butter prepared from pasteurised cream keeps better than butter prepared from soured cream, other conditions being equal.

Having determined the causes of rancidity, the next step was to find out the nature of the resulting compounds. A distillation of the rancid butter-fat in a current of steam has been found to show a very strong acidic, ketonic and aldehydic reaction. That is also the view of Solstein.² A determination of the molecular weight of the mixed fatty acids of the rancid fat was found to be 230 (Table No. 47), indicating an increased presence of lower fatty acids. Ketonic indications could be obtained in a very few samples which were thoroughly rancid and decomposed.³ The aldehydes present may be oenanthylic, pellarmonic, butyric or even caproic, the exact nature of which could not be determined with perfect certainty. In view of these facts enumerated above, it was thought necessary to find out some qualitative or quantitative method for detecting or determining rancidity respectively. A positive acetyl value has been found by the present authors to give clear indication of rancid nature of butter fat. No great value can be attached to the increase in the ester value which is no doubt attributable in the case of rancid fat to the presence of aldehydic products formed and to the decomposition of these into acids by the alcoholic potash. It will be of

(1) Schmalfuss, H. Werner and A. Gahrke *Fettchem. Umschau*, 1933, 40, 102-104.

(2) Solstein : *Chem. Rev. Fette U. Harz. Ind.*, 1905, 12, 117.

(3) Schmidt, J. S. C. I., 1898, 610 ; *Zeit. f. Anal. Chem.*, 37, 301, 1898 ; J. Mayrhofer. *Zeits. f. Untersuchung. d. Nahr-u-Genusem.*, 1898, 8, 552-53.

interest to note here that rancidity has been ascribed by some to the presence of fatty acids and esters,¹ a view not supported by recent investigations. Acid value has already been shown to be a very unreliable test for rancidity. The present authors, in conformity with the observations of Kerr and Sorber, have come to the conclusion that of all possible reactions for the qualitative and quantitative measurement of rancidity of butter fat, the acetyl value and the Kreis reaction,² the precursor of which is Epiphydrinic aldehyde are the best. Some discrepancies of Kreis reaction have been noticed in the case of very old samples of rancid butter fats, which have been explained by H. Ditz by saying that the negative Kreis test is a result of condensation of aldehyde with phloroglucinol under the influence of a strong acid. He found that the aldehydes gave a slightly coloured condensation product; the higher the percentage of aldehyde, the lower is the colour intensity and the greater is the amount of the precipitate. Again the lower the molecular weight of the aldehyde the more easily the condensation reaction takes place and consequently the more the disturbance of the colour. In this connection, the observations of Holm and Greenbank as well as T. W. Jones³ go to show that there is no relation between the degree of rancidity and the depth of colour in the Kreis reaction. That is also the experience shared by the present authors. Still the latter hold that Kreis reaction appears to be quite suitable as a qualitative and sorting test. It cannot be, of course, recommended with full authority, for studying the advancing process of rancidity. For this latter purpose, determination of acetyl value should be very reliable and it will

(1) C. Amthor; *Zeits. Anal. Chem.*, 1899, 38, I, 10-20.

(2) *Ind. Eng. Chem.*, 25, 167, 1933, See Ref. 2.

(3) *J. S. C. I.*, 43, 1258, 1924.

be worth while finding out some relation between the increasing rancidity and acetyl value for quantitative purposes. The latest index of rancidity proposed by Greenbank and Holm¹ should be given a fair trial before it can be accepted, as a universally correct method. A number of methods such as J. Bulir's,² Taufel and Thaler's reaction for ketone rancidity³ and the Taffel and Revis⁴ method have been devised for detection and measurement of rancidity. Every process for measuring rancidity which depends upon the general detection of aldehydes appears equally inaccurate. For this purpose, von Fellanberg⁵ has developed a method which depends upon the unusually sensitive colour reaction of Fuchsine with aldehyde. The disadvantage lies in the fact that the reagent is insufficiently defined and any attempt to use it as a quantitative reagent is uncertain.⁶ Other methods which have assumed a definite meaning based upon oxidisability of the liquids formed by the spoilage of fats or upon the water soluble decomposition products⁷ are also in vogue.

(1) Ind. Eng. Chem., Analyst, 2, No. 1. p. 9, 1930.

(2) B. G. A. 1926, 1, B66.

(3) Analyst, 1932, 57, 466.

(4) Analyst, 1931, 56, 323.

(5) Mitt. Lebensmittelunters. und. hygiene, 15, 98, 294, 1924.

(6) Zeit. unters Lebens, 32, 199, 1926,

(7) Zeit. Unters. Lebens 32, 195, 1926.

The following note by M.R.Coe, and J.A.Leclerc, on the photochemical studies of rancidity¹ is interesting.

“(1) The well known colour tests for rancidity and the peroxide test for the decomposition of an oil may not show conclusively that an oil is rancid. These tests are not reliable when applied to oils which have been properly protected from light. (2) Oils which have been protected from light with opaque black paper, or with green transmitted light delimited by 4900 to 5800-Å., remain free from rancidity even though they may have a peroxide value equal to or higher than an unprotected oil which has become rancid. (3) Similar results have been obtained when air has been bubbled through the oils at the rate of 6 litres per hour. (4) Oils protected from light as described have not shown any organoleptic rancidity even after a period of 7 months, although they gave strong positive tests with both Kreis and the von Fellenberg reagents, and also showed relatively high peroxide values. (5) A portion of the protected sample of the oil still free from organoleptic rancidity but having a peroxide value higher than that of an unprotected rancid oil was exposed to diffused day-light. By the end of 52 days, it had acquired a rancid odour and taste,

(1) *Ind. and Ing. Chem*, 1934, 26, 3, pp. 245-48.

Other tests for rancidity in fats are as follows :—

- (a) Barnicoat : *J. S. C. I.* 50, 361T, 1931.
- (b) Kilgore, L. B. : *Gil and Soap*, 10, 66, 1933.
- (c) King, Rosechen and Irwin : *Ibid*, 10, 105, 1933.
- (d) Lea : *Rept. of Food Investigation Board*, 1929, 30, *Proc. Royal Soc.* (London) 1088, 175, 1931.
- (e) Kreis Reaction by Powick, J., *Agr. Res.* 26, 323. 1923.
- (f) Browne, J. : *Am. Chem. Soc.*, 21, 975 ; 1899.
- (g) Heffter, A. : *Schweiz, woch. Chem. u.—Pharm.* 42, 320, 1904.
- (h) Taffel and Revis : *J. S. C. I.*, 50, 87T, 1931.
- (i) Wheeler, D. H. ; *J. Oil and Soap*, 9, 89, 1932.
- (j) Royce, H. D. : *Ind. Eng. Chem. Anal.* 5, 244, 1933.

while the original protected portion of the same sample remained free from rancidity. (6) In the case of cotton-seed and corn-oils, the results of these experiments support the view that oxidative rancidity may be due principally to photochemical action of light on a compound which probably exists simultaneously in the oil or is produced from the compounds which give rise to the formation of peroxides."

The disadvantages of these methods lie in the fact that a normal fat always gives a certain oxidation number which increases with the spoilage, so that it is difficult to show a limit when the fat is strongly rancid and when the liquid aldehydes are oxidised to the acids and the oxidation number is lowered.

The craze for such handy and so-called test-tube methods no doubt has come to be very popular, though very unfortunately, even among chemists. The scientific observations do not give any encouragement of a very authoritative nature to these ideas. The present authors have tried a good many of such methods and found them unsatisfactory. Their final conclusions are in entire agreement with that of the German Commission which refused to recognize any rancidity-tests not based upon taste and odour.¹

A further study of the methods recommendable to get rid of rancidity in the spoilt fats was taken up. The products held responsible for the odour and taste rancidity are all invariably of very volatile nature and they are mostly also water soluble. Therefore, the method proposed and also adopted successfully is to volatilise all such rancidity-bearing compounds by injecting low pressure steam into the sample under refinement, separating all

(1) Davidsohn : Chem. Ztg. 54, 606, 1930.

condensed water, dehydrating the fat completely by means of anhydrous sodium sulphate, filtering and finally storing in thoroughly sterilized containers of suitable metal devoid of all air and moisture, and kept preferably in a cold and dark place. Addition of salt is helpful in preservation. The original aroma and taste may not be recovered although the fat is rendered thus quite edible. To regain the fresh butter aroma, the modern perfumer has come forward with his offer of a synthetic butter aroma which may be added to advantage.

In Europe, a similar problem has been solved with regard to the manufacture of renovated butter as follows :—¹

“The butter fat is separated from the rancid butter by melting and separation from the aqueous solution and the curd. The fat is next blown with air to remove the objectionable flavour and then quickly cooled in a current of cold water so as to prevent the separation of the more liquid portion of the butter from the more solid portion. The butter-fat is then churned with fresh milk to which cultures of a particular bacteria have been added. The milk soon becomes sour and coagulates, thus furnishing an artificial curd containing about the same proportion of nitrogen as that of the curd of genuine butter.”

Finally, a study has been made of the action of different metals on butter-fat. In India, the fat is generally stored either in aluminum, iron, tinned copper, or tinned utensils as well as in earthen jars. Of all these the latter is the most harmless and positively helpful in preserving the contents. A perusal of tables from Nos. 20 to 27 prove very conclusively that aluminium spoils the fat

(1) J. Oudsteyn, United States Patent, 1012 471 ; American Pharm. Products Co., Eng. Patent 7500, 1907 ; Higgins, Patent 15221, 1913.

to a very little extent, while copper does very great harm, rendering the fat not only green but also poisonous for human use. Next to the use of the gift from Mother Earth comes the place of iron which from time immemorial has been in every home for the storage of butter fat in India.

As "Prevention is better than cure", it will not be out of place to suggest in view of the present study the best method for preservation of the fat. The procedure recommended is :—

1. Complete elimination of the non-fatty ingredients like casein and milk-sugar by simple filtration.

2. Complete dehydration by the use of freshly prepared anhydrous sodium sulphate or alum.

3. All the containers(either of earthenware or of metals like iron or aluminium) and apparatus etc., employed should be thoroughly cleansed and sterilized before use.

4. Regarding sterilization of the properly filled containers at a suitable temperature capable of killing all bacteria, Zoffmann¹ has shown that some fungi² are not killed at those temperatures at which oils and fats are prepared. Nieloux³ showed that cotton-seed cytoplasm which under suitable conditions causes rapid hydrolysis of oils and fats, if suspended in oil, may be heated for two hours upto a temperature of 100°C. or for 15 minutes to 110°C. without losing its fat-hydrolysis power. Micro-organisms have been found in poppy-seed oil.⁴

(1) Chem. Res. 1904, 7.

(2) Dixon: Nature, 1909, Nov. p. 100, cp. also J. White Proc. Roy. Soc. 81, 417.

(3) Contribution a l'etude de la saponification des corps gras, Paris, 1906, p. 30.

(4) Kirschner, Berichte der botan. Gesellschaft, 1888, 10.

5. Addition of salt as a preservative, or Benzoic acid and Sodium Benzoate also give satisfactory results.¹

6. Storage in a damp-proof cold and dark place is recommended.

In view of the above mentioned data, liquid fats, in the opinion of the present authors offer a greater action surface because of their fluidity to the attack of rancidifying agents and hence they turn rancid more readily than solid ones. That may be the reason why butter fat gets spoilt more readily in summer than in winter. Glycerol as such does not seem to remain free during rancidification. It is likely that the hydrolysed glycerol forms glycolic acid and an aldehyde. Again the higher the contents of non-volatile insoluble and unsaturated fatty acids, the easier appears to be the process of rancidification. The higher the proportion of insoluble saturated fatty acids and the lower the percentage of unsaturated glycerides in the fat, the less seem the chances of rancidity. Perhaps this explains the good keeping properties of coconut oil and tallow as compared with butter fat.

In all cases of rancid samples of butter fat, an acrid and burning taste with an unpleasant aldehydic aroma develops characteristically. It is very difficult to exactly define these properties ; still even a little practice enables any one to detect this change due to rancidification.

(1) C. Thorn, *Journ. Agric. Res.* 1915, 3, 301, cp. also R. Washburn and A. Dahlberg, *Journ. Dairy Sci.* 1917, 1, 114, and W. Combs and C. Eckles, *ibid*, 1917, 1, 347 ; also O. Pattick, *Journ. Agric. Res.* 1908, 11, 22, 32 ; *Zentralbl. f. Bakterien u. Parasitenkunde*, 1908, 11, 22. cp. also D. C. Dyer, *Journ. Agric. Res.* 1916, 6, 927 ; Bemolmans, *Zeits. f. Unters. d. Nahrsg. u. Genussam.* 1907, XIII, p. 492 ; Grimaldi, *Chem. Zeit.*, 1908, 794 ; K. B. Lehmann, *ibid*, 1908, 949 and 1911, 1297 ; Polenske, *Arb. a. d. Kaiserl. Gesundheitsamtes*.

Conclusions.

1. The fundamental and primary cause of rancidity in butter fat is the action of moisture liberating free-fatty acids and glycerol. Air comes next to complete the process of rancidification.

2. There are two distinct stages in the development of rancidity : (a) odour-rancidity brought about by the hydrolytic action of the moisture and (b) taste rancidity brought about by the oxidising action of air on the unsaturated part of the fat and decomposed products. A completely rancid sample of the fat possesses both.

3. Moisture and air together act simultaneously, either of these alone being unable to produce rancidity.

4. Light, casein, milk-sugar, micro-organisms and bacteria etc., act as positive catalysts to hasten the process of rancidification.

5. The rancid behaviour of butter fat is revealed by the development of the characteristic aroma and taste, increase in acid value and the observation of the Kreis reaction. A positive acetyl value test is dependable from the chemical point of view.

6. The rancid butter fat can be revived by the process mentioned in the previous pages of this chapter. Such samples, of course, cannot exactly equal the freshness of an unspoilt butter fat.

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